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TEMPERATURE RELATIONS OF ELEVEN SPECIES OF RHIZOPUS [1]

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INTRODUCTION

Harter, Weimer, and Lauritzen (*12*) [2] have recently shown that the typical softrot of sweet potatoes may be produced by nine different species of the genus Rhizopus. It has been demonstrated likewise that these fungi can produce a similar decay of a large number of fruits and vegetables when the host and parasite are brought together under suitable conditions (*11*). The importance of temperature as one of the conditions essential for infection by these fungi was pointed out, it having been found necessary in all cases to expose the hosts to temperatures within the range most suitable for the growth of the fungus in order to obtain infection. These fungi were placed roughly into high, low, and intermediate temperature groups. More recent studies have been made [3] in which the temperature limits for growth of these fungi upon the sweet potato have been determined. The behavior of these fungi upon the living host is of special interest, since it is under these conditions that they become of economic importance. However, in studies of this nature two living organisms are involved, each of which may respond quite differently to various conditions of the environment. At high temperatures the physiological activities of the host are accelerated, while the reverse is true at low temperatures. The growth of the fungus will likewise be stimulated or retarded, depending upon the temperature employed; consequently any living host as a medium for testing the response of the species of Rhizopus to temperature would not be uniform at different temperatures and would therefore be unsatisfactory. It was the object of these investigations to determine the influence of temperature on the development of 11 species of Rhizopus when grown upon an artificial culture medium of uniform composition.

The studies, the results of which are presented below, include the effect of temperature first on the germination of the spores, second on the growth of the mycelium, and third on the fruiting of the fungi.

SPECIES STUDIED

The species of Rhizopus studied were the same as those used in former investigations: Namely, *nigricans* Ehrnb., *reflexus* Bainier, *chinensis* Saito,

[1] Accepted for publication May 29, 1922.
[2] Reference is made by number (italic) to "Literature cited," p. 39-40.
[3] By J. I. Lauritzen, of the Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture. The results have not yet been published.

tritici Saito, *artocarpi* Racib., *delemar* (Boid.) Wehmer and Hanzawa, *maydis* Bruderl, *nodosus* Namysl, *oryzae* Went and Pr. Geerligs, *microsporus* v. Tieg., and *arrhizus* Fischer. As previously explained (*12*) all the original cultures except those of *nigricans* and *arrhizus* were obtained from Mr. E. D. Eddy. Each organism was pure-lined by isolation from a single spore. A comparison with the original descriptions of each species showed no appreciable disagreement.

METHODS

In order to keep the stock cultures in a vigorous state of growth they were renewed about once each month by transferring to sweet potato agar in small Erlenmeyer flasks and incubated at a temperature of from 22° to 26° C. Cultures of from 5 to 10 days old were used in the experiments. Preliminary investigations showed that Rhizopus spores germinate more readily in some good nutrient medium such as sweet potato decoction than in water, hence the former medium made as previously described (*10*) was used.

In order to have available a solution of uniform composition for all the spore germination tests, a large volume of sweet potato decoction was prepared and stored in small flasks stoppered with cotton and covered with several thicknesses of oiled paper to retard evaporation. Each flask contained about 120 cc. of the decoction, which was about the amount required for the study of the spore germination of each organism. By storing the solution in small flasks the necessity for resterilizing the stock solution each time some of it was removed and thereby possibly changing its chemical composition was avoided. The loss by evaporation was restored by bringing the solution up to its original volume by the addition of distilled water. A loop of a spore suspension made in this medium was placed upon a clean cover slip, which was then sealed with vaseline in an inverted position to a glass ring cemented to a slide with a mixture of beeswax and vaseline. In the bottom of a cell so constructed was placed a small quantity of the same solution. The cells used were comparatively large (13 mm. high by 16 mm. in diameter) and provided sufficient air for the germinating spores.

In view of the fact that some investigators have found the hanging-drop method of studying spore germination unsatisfactory, the writers made several preliminary comparative tests of different methods. These showed that so far as these species of Rhizopus are concerned as reliable results could be obtained by the hanging-drop method as by any of the others, as, for example, in small volumes of solution in test tubes. Furthermore, since repeated observations must necessarily be made to determine accurately the time of germination the hanging-drop method lent itself more readily to manipulation than the test-tube method in which a fresh mount must be made for each observation. The hanging-drop cultures were placed in the incubators as soon as possible after they were prepared. The incubators were manufactured by Paul Altmann, were electrically controlled, and ranged in temperature from 1.5° to 50° C. Usually the temperatures in the individual chambers remained almost constant, varying for the most part less than a degree except in the lower ones, where there was sometimes a variation of 2° or 2.5° C. Pans of water were kept in the bottom of the chambers to keep the air moist. At least four and more often eight hanging drops were used at each temperature.

The rate of growth of these fungi at various temperatures was studied by growing them in Petri dishes containing 10 cc. of a 2 per cent Irish potato agar without additional sugar. A small drop of a suspension of spores in water was placed in the center of each plate with a 2-mm. platinum loop. The plates were placed in the incubators as soon as they were prepared, and the rate of growth was determined by measuring twice daily the diameter of the mycelial felt formed. Five Petri dishes were placed at each temperature. When the results were not entirely satisfactory the experiment was repeated.

The effect of temperature on sporangia formation was determined by growing the fungi at different temperatures in 100-cc. Erlenmeyer flasks on 30 cc. of 2 per cent Irish potato agar and observing the temperatures at which the sporangia were produced and the time required for their formation.

INFLUENCE OF TEMPERATURE ON SPORE GERMINATION

The effect of temperature upon spore germination has been studied by a number of investigators and by several different methods. The percentage of germination at different temperatures has been used as a measure of the influence of temperature upon spore germination by Melhus (*14*), Doran (*4*), Reed and Crabill (*18*), and others. Naturally the method used in any particular investigation must depend upon the type of data desired. Preliminary tests showed that practically 100 per cent of the spores of *Rhizopus* spp. germinated except at extreme temperatures; hence, the percentage of germination could be used as a measure of the effect of temperature only by taking into consideration the time factor. The number of spores which germinate and cause infection in the case of decay-producing fungi of this type is not of as much importance as it is in the case of organisms producing other types of diseases, such as leafspots. In the former case a host may be completely destroyed as a result of a single infection, while in the latter the total amount of damage done often depends upon the number of individual infections. In view of these facts the writers decided to use the time required for germination to begin as the measure of the influence of temperature upon this phenomenon. Anderson (*2*), Melhus (*14*), Ames (*1*), Rands (*16*), Ravaz and Verge (*17*), Shapovalov (*19*), and others have used this same criterion in their investigations. However, in no case is it made clear when these writers considered germination to have taken place. It is possible to use as a criterion the time when the maximum percentage of the spores have germinated or when the spores which germinate first have produced germ tubes. With Rhizopus a few spores in each drop produce a germ tube first, followed very soon by others, the number gradually increasing so that in a short time nearly 100 per cent of the spores have germinated. The period intervening between the time when the germ tubes appear on the spores which are the first to germinate and the time when all the spores have germinated varies with the temperature, being shortest at the optimum and gradually increasing as the upper and lower temperature limits are approached. As a criterion of germination the writers decided to use the time necessary for the spores which germinate first to produce a germ tube equal in length to the diameter of the spores. Using the figures thus obtained, curves were plotted which show the variation in time due to the difference in temperature. The time necessary for the germ tubes to reach some specific

number of microns in length might have been employed; but the measure selected was easier to determine, since the spore was always present with the germ tube for quick comparison, while considerable time would have been required to bring the spore in line with the micrometer scale for measurement. In these studies time was a very important factor, since germination often occurred in three or four incubators at nearly the same time, often not over from 5 to 15 minutes apart. Some care had to be exercised, especially at the less favorable temperatures, as often one or a very few spores would start to germinate considerably in advance of the remainder. However, by examining the hanging drop cultures frequently it was possible to determine with a fair degree of accuracy the point sought. Slides were examined near the incubators and were kept out usually less than a minute. Only at the higher temperatures was there any appreciable fluctuation as a result of opening the doors, and in such cases the normal temperature was quickly restored.

The results obtained from the study of the germination of the spores of 10 of the species is shown by the curves in figure 1. The germination of *Rhizopus maydis* spores was not studied for the reason that under the conditions under which the cultures were grown spores were not produced in sufficient quantity to carry out the experiment.

In the curves in figure 1 the time in hours necessary for the germ tubes to reach the length of the diameter of the spores was plotted on the abscissa, while the temperature in degrees centigrade was plotted on the ordinate. The maximum temperature for germination as indicated by the table is that temperature at which no germination took place. The temperature at which germination will just take place is very difficult to determine, since at 1° or 2° C. below the maximum it frequently starts and then stops before the germ tube reaches the diameter of the spore. For example, *Rhizopus artocarpi* spores germinated readily at 32.3° while at 33.4° only about one-half of 1 per cent of the spores sent out germ tubes, which finally reached a length equal to twice the diameter of the spore and then stopped. At 34.5° the spores became somewhat swollen, which is a condition always preceding the extrusion of the germ tube. After 48 hours the spores had been killed at the two higher temperatures, as indicated by the fact that they failed to germinate when placed at a temperature favorable for germination. Similar results were obtained with other species. No attempt was therefore made to establish a definite maximum, but it may be said that in general it is somewhere from 1° to 2° lower than the temperature plotted as being the inhibiting temperature. In every case where there was no germination within 24 to 48 hours the spores were found to have been killed. The temperatures just above the maximum which inhibited germination of the spores of the different species are as follows: *artocarpi*, 34.5°; *tritici*, *delemar*, *oryzae*, *nodosus*, and *arrhizus*, 45.5°; *chinensis*, 52°; *reflexus*, 38°; *nigricans* and *microsporus*, 34°. The lower temperature limits for germination for only two of the species, *nigricans* and *microsporus*, are shown by the curves, since in all other cases the time for germination at the minimum temperature was more than 100 hours. The spores of *chinensis*, *oryzae*, and *delemar* had not germinated during 30 days' exposure at 8.5°, 7°, and 7°; but germination did take place in that length of time at temperatures of 10°, 9°, and 8.7°, respectively. On the other hand, the spores of the remaining species, *reflexus*, *arrhizus*, *tritici*, *nodosus*, and *artocarpi*, germinated at 1.5°, the lowest temperature tried, in 5, 15, 22, 14, and 6 days, respectively. The percentage of spore germination at

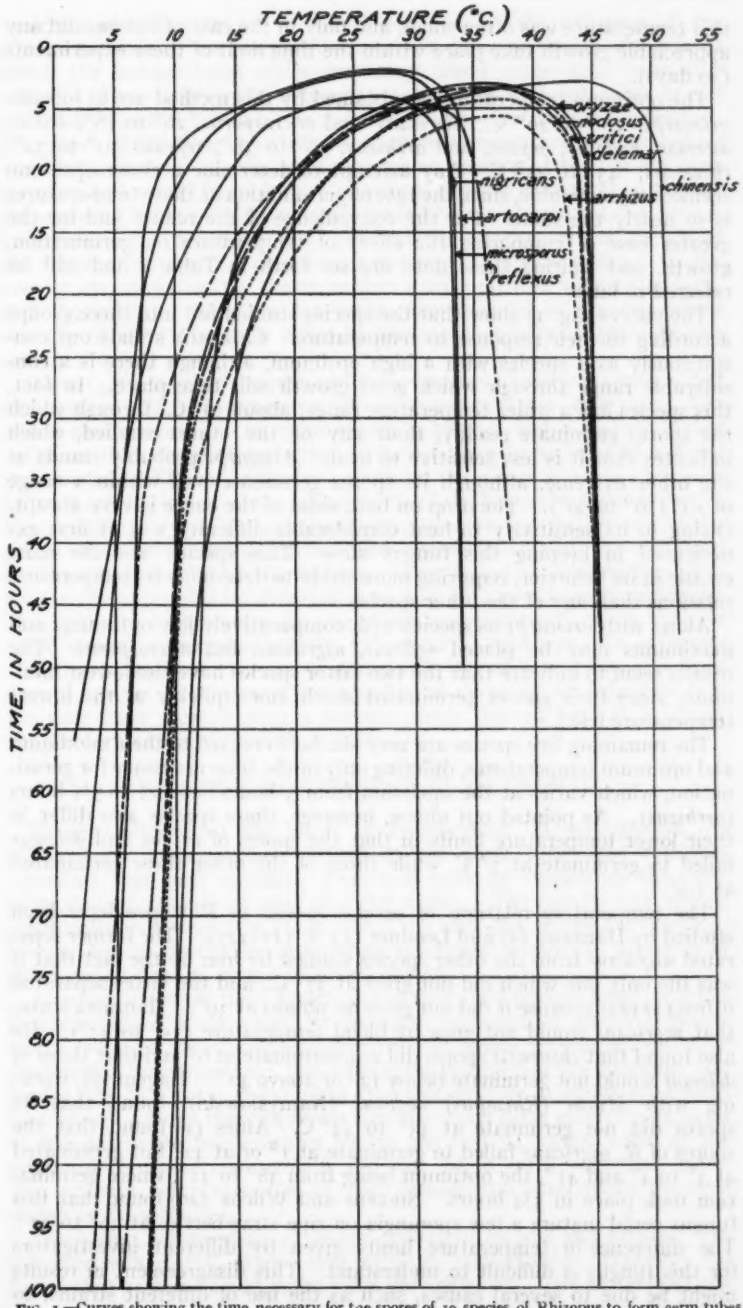

FIG. 1.—Curves showing the time necessary for the spores of 10 species of Rhizopus to form germ tubes equal in length to the diameter of the spore at different temperatures.

this temperature was often small, and only in the case of *reflexus* did any appreciable growth take place within the time limit of these experiments (30 days).

The optimum temperatures as obtained by this method are as follows: *artocarpi*, 26° to 29° C.; *nigricans* and *microsporus*, 26° to 28°; *tritici*, *delemar*, *nodosus*, *oryzae*, and *arrhizus*, 36° to 38°; *reflexus* 30° to 32°; *chinensis*, 43° to 45° C. Any attempt to determine a closer optimum seemed impracticable, since the rate of germination at these temperatures is so nearly the same. For the convenience of the reader and for the greater ease of comparing the effect of temperature on germination, growth, and fruiting these data are set forth in Table I and will be referred to later.

The curves (fig. 1) show that the species studied fall into three groups according to their response to temperature. *Chinensis* stands out conspicuously as a species with a high optimum, although there is a considerable range through which good growth will take place. In fact, this species has a wider temperature range, about 30° C., through which the spores germinate readily, than any of the others studied, which indicates that it is less sensitive to heat. *Artocarpi* probably stands at the other extreme, although its spores germinate well within a range of 20° (10° to 30°). The drop on both sides of the curve is very abrupt. Owing to its sensitivity to heat considerable difficulty was at first experienced in keeping this fungus alive. This species was the most erratic in its behavior, requiring more study to determine its temperature relations than any of the other species.

Along with *artocarpi* as species with comparatively low optimums and maximums may be placed *reflexus*, *nigricans*, and *microsporus*. The results seem to indicate that the two latter species have the lowest minimum, since their spores germinated much more quickly at the lowest temperature tried.

The remaining five species are very similar in regard to their maximum and optimum temperatures, differing only in the time necessary for germination, which varies at the optimum from 3 hours (*oryzae*) to 5½ hours (*arrhizus*). As pointed out above, however, these species also differ in their lower temperature limits in that the spores of *oryzae* and *delemar* failed to germinate at 7° C. while those of the other three germinated at 1.5°.

The temperature relations of several species of Rhizopus have been studied by Hanzawa (*9*) and Lendner (*13*, *p. 111–127*). The former separated *nigricans* from the other species studied by him by the fact that it was the only one which did not grow at 37° C., and the latter separated it from *oryzae* because it did not grow on potato at 39°. Hanzawa states that *nigricans* would not grow at blood temperature (35° to 37°). He also found that *chinensis* spores did not germinate at 6° and that those of *delemar* would not germinate below 12° or above 42°. Hagem (*8*), working with *Mucor* (*Rhizopus*) *nodosus* (Namyslowski), found that its spores did not germinate at 43° to 44° C. Ames (*1*) found that the spores of *R. nigricans* failed to germinate at 1° or at 42° but germinated at 3° to 4° and 41°, the optimum being from 38° to 41°, where germination took place in 5½ hours. Stevens and Wilcox (*20*) found that this fungus could mature a few sporangia on ripe strawberries at 36° to 37°. The difference in temperature limits given by different investigators for this fungus is difficult to understand. This disagreement in results might be due to several causes, such as the use of different strains, to

incorrect identification or to mixed cultures, to the influence of the culture media, etc. A comparative study of different strains of *nigricans* which the writers have under way may throw some light upon this subject. The variation in temperature between different parts of an artificially heated incubator is a factor which can not be overlooked. The upper strata of air in an incubator whose temperature is measured by means of a thermometer inserted through an opening at the top has been found to be in some cases from 1 to several degrees warmer than that at the center or bottom. In chambers heated by a water jacket the writers have recorded a difference of 5° between the temperature of the air at the top and bottom of a chamber, a distance of only 16 inches. In the present investigations the thermometers were compared with a standardized thermometer and then the bulbs were lowered to the immediate vicinity of the cultures in order to obviate as nearly as possible this source of error.

Dunn (5) found that the plus and minus strains of *nigricans* with which she worked differed somewhat in their temperature relations. The minus strain seemed to be more vigorous, since it had a slightly higher optimum than the plus and grew at both a slightly higher and a lower temperature. The optimum for growth by the minus strain lay between 25° and 28° C., which agrees closely with that obtained by the writers—approximately 25°. The maximum temperature for the growth of the minus strain was about 31°, which also agrees closely with that obtained for the strain studied by the writers. Dunn found a considerable difference in the optimums for different strains of Rhizopus from strawberries. The optimum temperature for one strain was about 36°, while that for other strains was approximately 27° or 28°. However, she expresses doubt as to whether she was working with strains or species, and since several species of Rhizopus are capable of decaying strawberries (*11*) she may have worked with a species which thrived best at a higher temperature rather than with a strain.

INFLUENCE OF TEMPERATURE ON GROWTH

Having determined the effect of temperature on germination, its influence on the continued growth of the germ tubes and resulting mycelium was next studied. This was done by measuring the daily increment of growth on agar in Petri dishes. The results are shown graphically in figures 2 to 12, in which the base line shows the temperature in degrees centigrade and the perpendicular shows the diameter of the mycelial disks at stated intervals of time.

An examination of these figures shows that they possess some common characteristics. Each series, for example, originates at scattered points to the left (the lowest temperatures tried at which growth did not take place within the given time), then rises in the direction of the optimum, and finally falls to a point at the right (the temperature at which no growth took place). From these figures it is evident that some growth took place at the maximum temperatures during the first 24 hours, while at the minimum temperatures growth did not begin until sometime later. The optimum temperature in all cases remains the same within the time shown in these graphs.

The lowest temperature at which growth of *maydis* and *arrhizus* was observed was 7.4° C. after 7 and 12 days, respectively. Hanzawa (*9*) found that *arrhizus* could make some growth at 6°. The writers did not

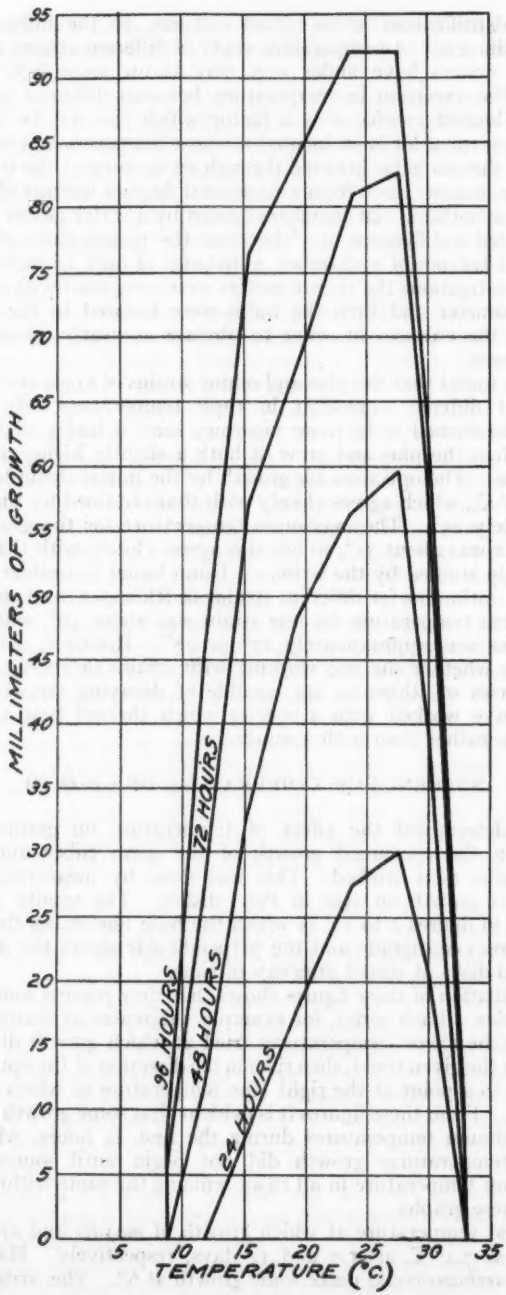

FIG. 2.—Graph showing the rate of growth of *Rhizopus artocarpi* at different temperatures.

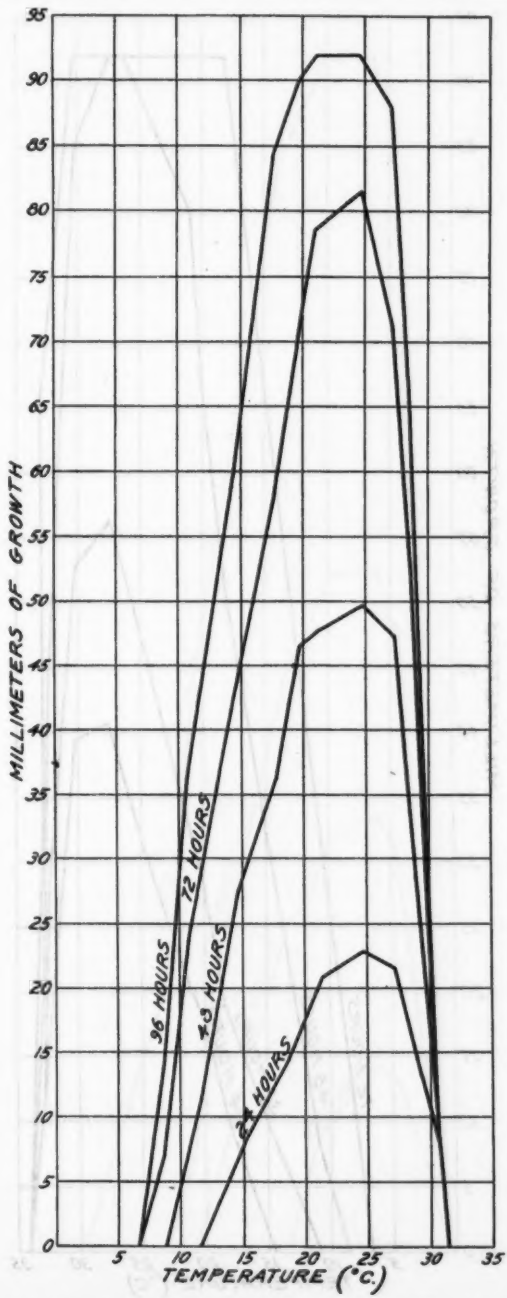

FIG. 3.—Graph showing the rate of growth of *Rhizopus nigricans* at different temperatures.

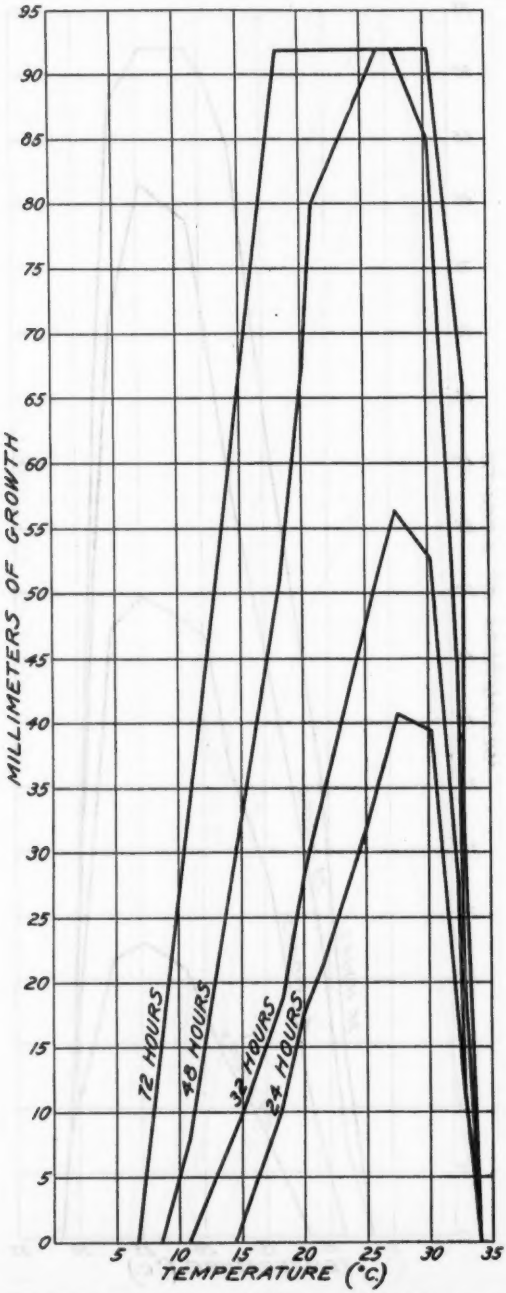

FIG. 4.—Graph showing the rate of growth of *Rhizopus reflexus* at different temperatures.

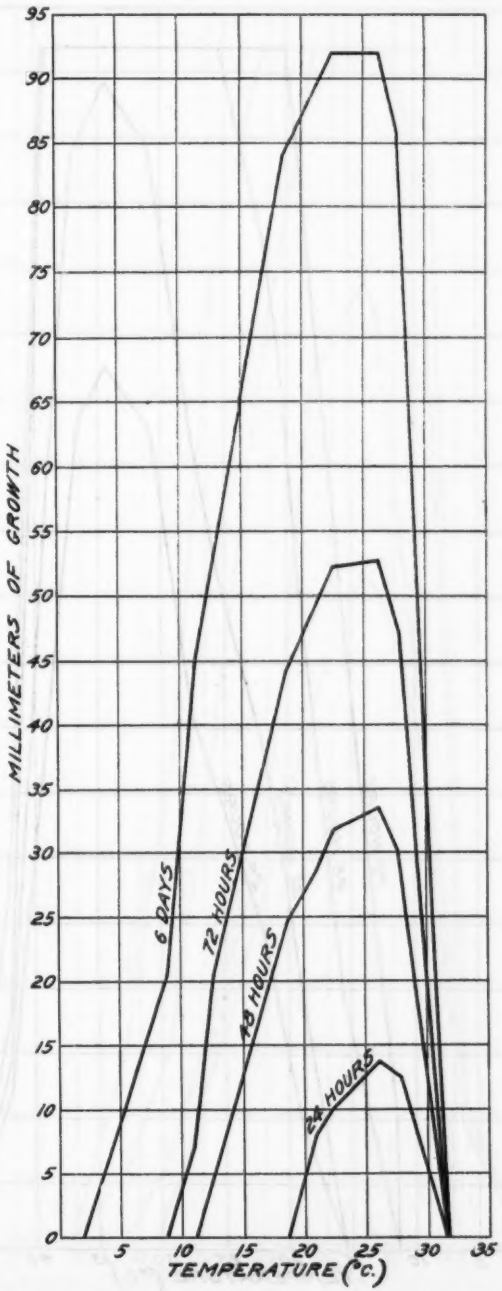

FIG. 5.—Graph showing the rate of growth of *Rhizopus microsporus* at different temperatures.

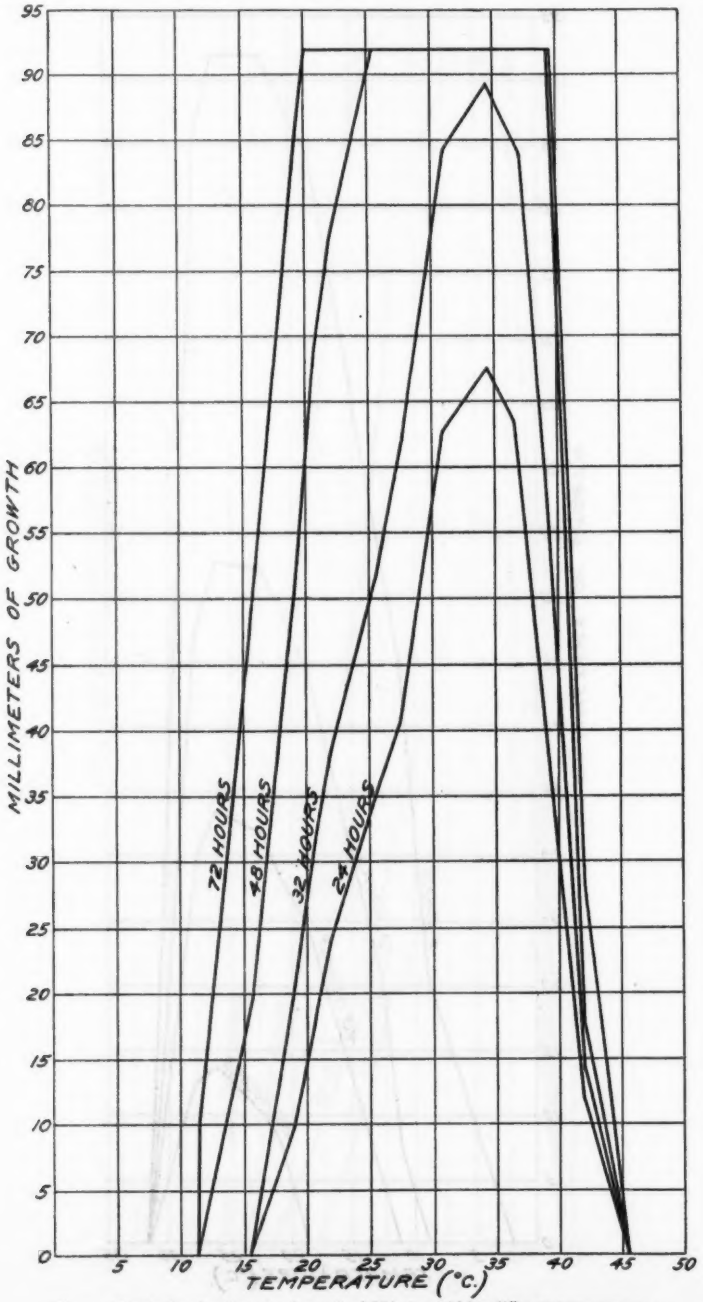

FIG. 6.—Graph showing the rate of growth of *Rhizopus tritici* at different temperatures.

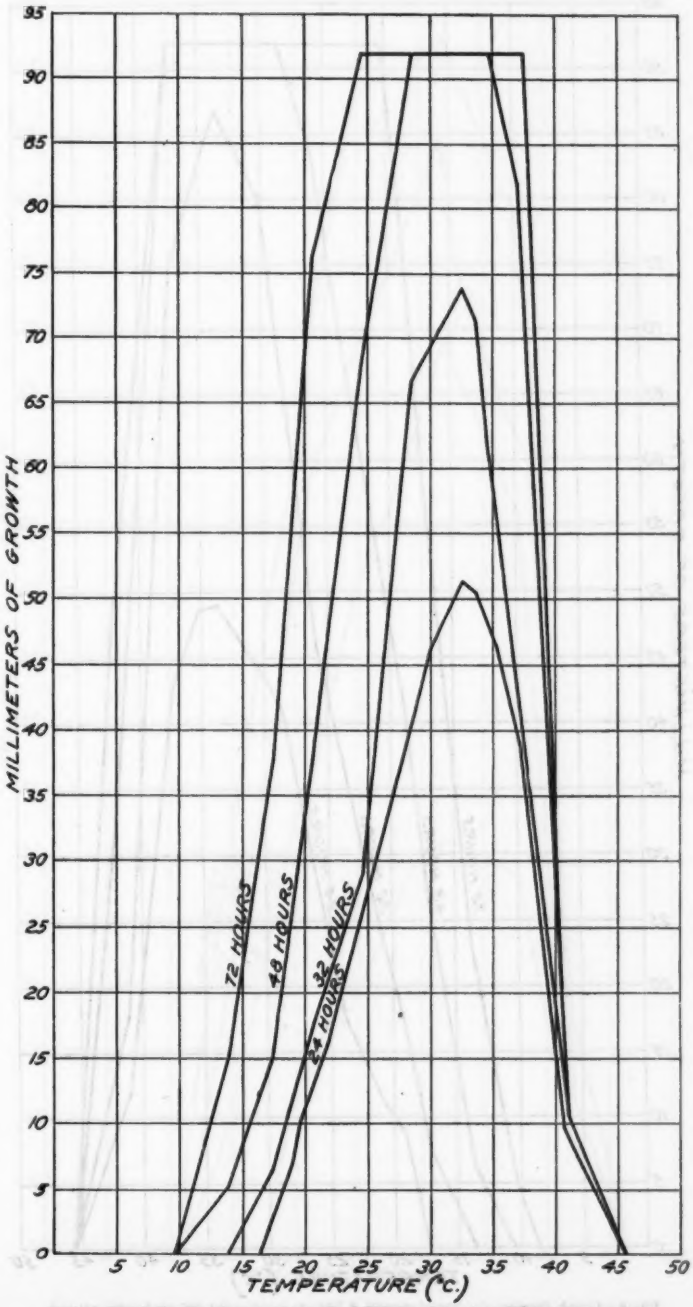

FIG. 7.—Graph showing the rate of growth of *Rhizopus delemar* at different temperatures.

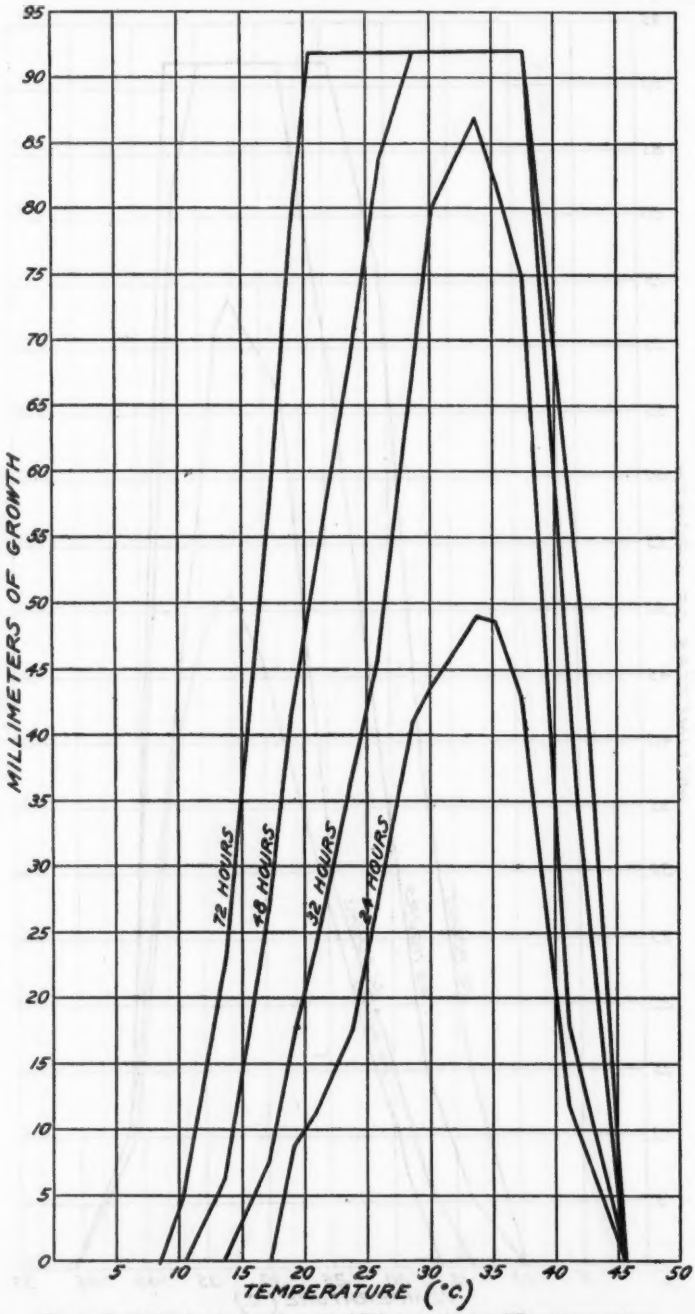

FIG. 8.—Graph showing the rate of growth of *Rhizopus nodosus* at different temperatures.

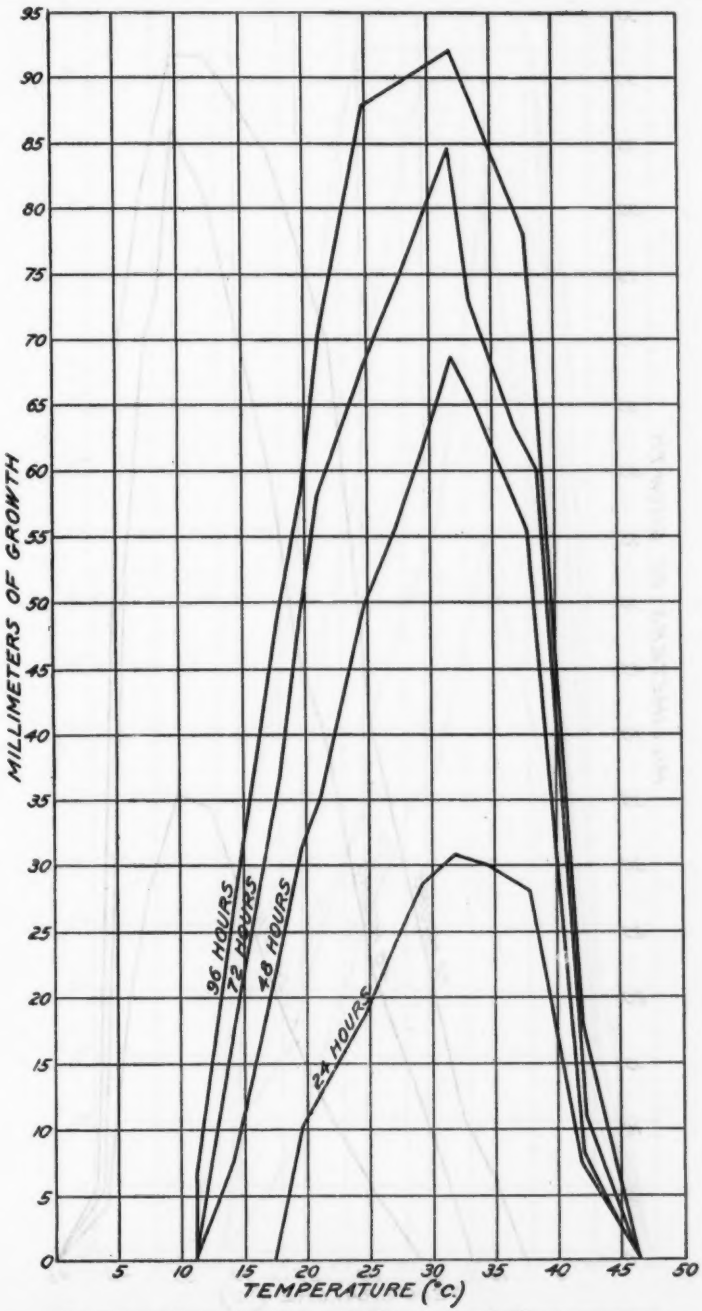

FIG. 9.—Graph showing the rate of growth of *Rhizopus oryzae* at different temperatures.

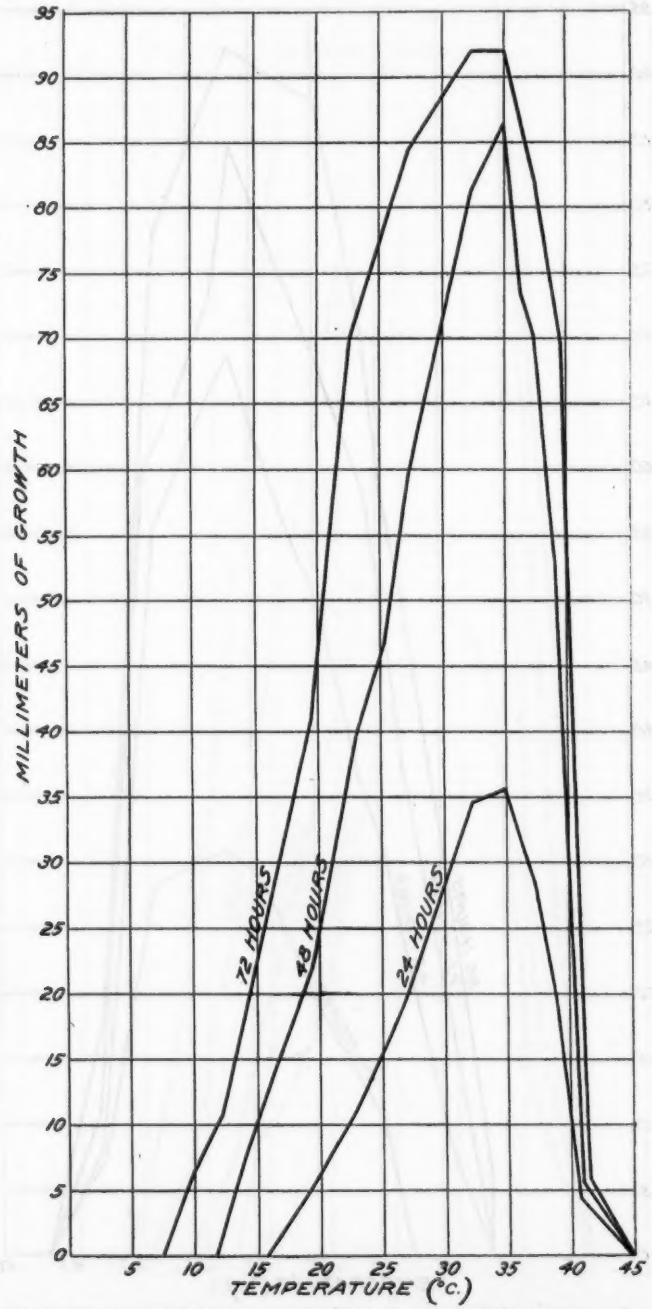

FIG. 10.—Graph showing the rate of growth of *Rhizopus arrhizus* at different temperatures.

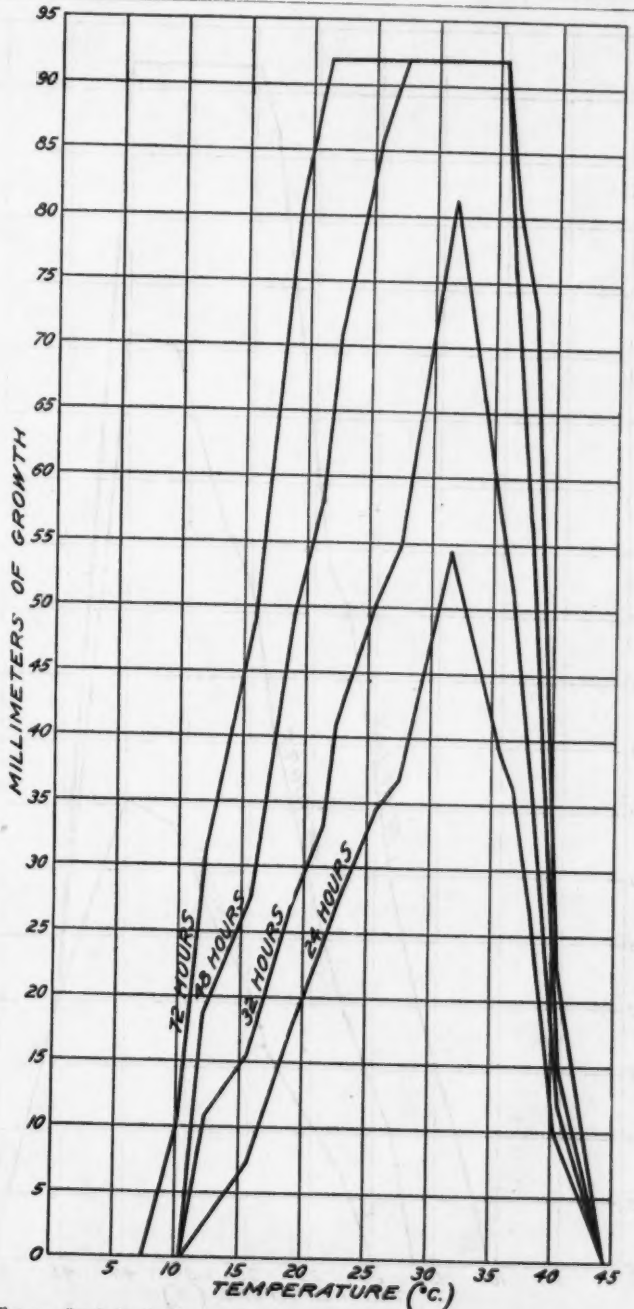

FIG. 11.—Graph showing the rate of growth of *Rhizopus maydis* at different temperatures.

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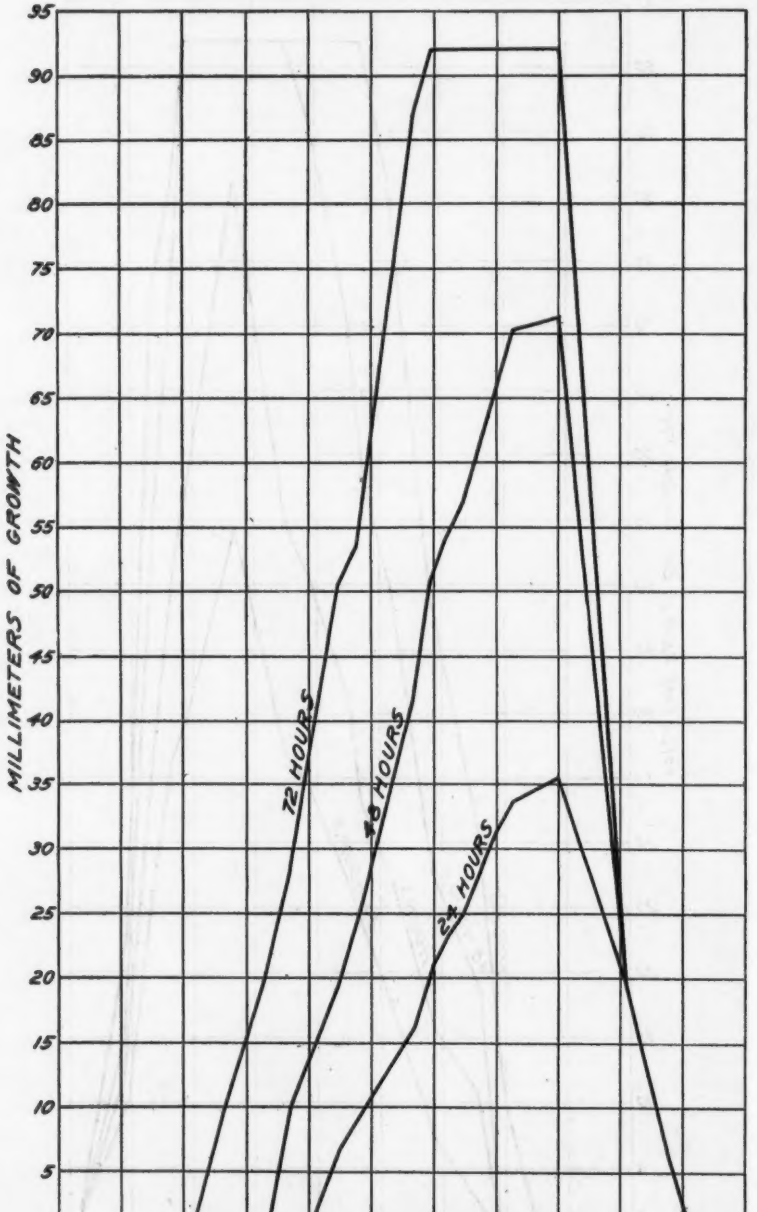

FIG. 12.—Graph showing the rate of growth of *Rhizopus chinensis* at different temperatures.

try a temperature between 7.4° and 1.5°, but it is quite probable that growth will take place below 7.4° if sufficient time is allowed, since the spores germinated at 1.5°. Growth by *tritici*, *nigricans*, and *nodosus* at 6.5° began after 12, 8, and 10 days, respectively. These fungi also made some growth at 6° to 8° in Hanzawa's experiments. *Microsporus* and *reflexus* are the lowest temperature forms so far as growth is concerned. They began to make an appreciable growth at 1.5, the lowest temperature tried, after 10 days. *Artocarpi*, a low-temperature form as indicated by its optimum, made some growth at 9° but none at 7°, while *chinensis*, which has a high optimum, made some growth at 10.4° but none at 7.5°; in other words, growth was inhibited at practically as high a temperature in the low- as in the high-temperature form. At 11° and 9.8° *oryzae* and *delemar*, two forms very closely related both morphologically and physiologically, made some growth in 4 and 16 days, respectively. However, at 7.5° growth was entirely inhibited. *Oryzae* was not subjected to any temperature between 11° and 7.5°, so it is possible that some growth might have taken place at a temperature somewhat below 11°. Hanzawa found that this species made some growth at 6°, while the upper and lower limits for *delemar* were found to be 12° and 42°, respectively. It may be said, then, that the minimum temperature for growth varies with the time for the first 5 to 15 days, depending upon the species. After that time the true minimum—that is, the lowest temperature at which growth will take place regardless of the time—is reached. Thus growth decreases as the temperature is lowered until a point is reached where it is entirely inhibited. However, cultures which failed to grow after 30 days at the inhibitive temperature or lower develop rapidly when transferred to a more favorable temperature, showing that the protoplasm of the spore has suffered no harmful effects. These results are in general accord with those of other workers with other organisms.

It was pointed out above that there was visible growth at the maximum temperature by the end of the first 24 hours in each species. It would appear from the graphs that the maximum temperature in all cases is fixed—that is, that it does not change during the course of the experiments as does the minimum. However, this is not the case. *Delemar*, for example, made a growth about 9 mm. in diameter at 41° C. in the first 24 hours, which reached 10.5 mm. during the following 24-hour period and then ceased altogether, the mycelium failing to grow when placed at a favorable temperature. In this case the maximum for the first 48 hours was about 41°, after which even at 38° the growth was considerably retarded although not inhibited within the time limit of this experiment. Shifting of the maximum was noted with the other species. No doubt if temperatures sufficiently close were tried over a considerable length of time and by a method sufficiently delicate to determine accurately very small increments of growth a so-called shifting of the maximum would be found in all cases. As the temperature rises above the optimum a retarding soon followed by an inhibiting effect is noted. A comparatively short exposure to the inhibiting temperatures results in the death of the organism, although it is possible that a temperature slightly below the maximum established might be found which would inhibit growth for a considerable length of time and yet would not kill the fungus. Fawcett (*6*) has found a similar shifting of the maximum. Shifts from 36° to 31°, 38° to 35°, 46° to 35° were noted in *Pythiacystis citrophthora* Smith and Smith, *Phytophthora terrestria* Sherbakoff, and *Diplodia natalensis* Evans, respectively. All of these fungi

showed a lowering of the apparent optimum of from 3.5° to 6°. *Phomopsis citri* Fawcett, however, did not show similar changes. No such change in the optimum for growth of the species of Rhizopus studied by the writers was noted. It is possible that if the fungi had been grown for longer periods of time some such shifting of the optimum would have

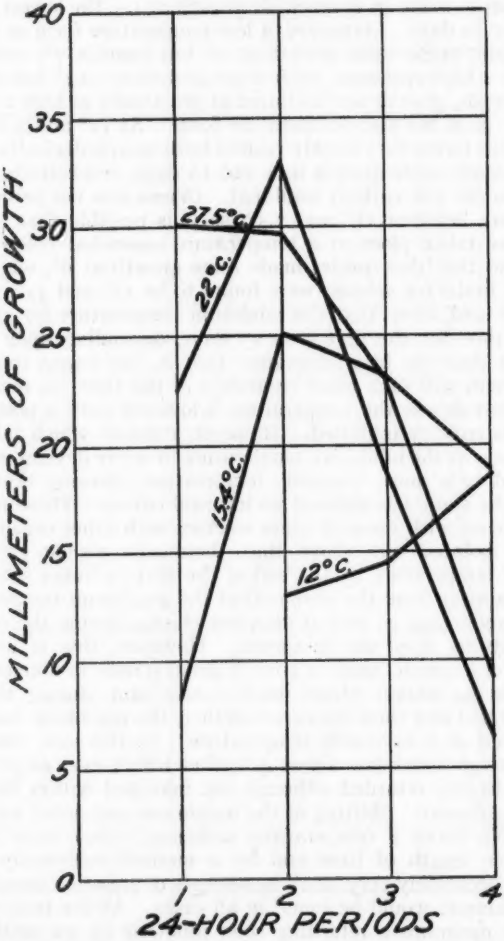

FIG. 13.—Graph showing the increase of growth of *Rhizopus artocarpi* for consecutive 24-hour periods.

become apparent. Hanzawa (*9*) found that the cardinal temperatures for some of these fungi were as follows: *Delemar*, *nigricans*, *arrhizus*, and *nodosus* have maximums of 42°, 35° to 37°, 42°, and 43° to 44°, respectively; *oryzae*, *tritici*, and *chinensis* all made a good growth at 38° to 42°; while the optimums for *delemar*, *oryzae*, and *tritici* were 30°, 30° to 40°, and 30° to 35°, respectively. Hagem (*8*), on the other hand, found that

nodosus, *nigricans*, and *arrhizus* had for their upper limits 43° to 44°, 33.5°, and 42°, respectively; Lendner (*13*) gives 30° to 35° as the optimum for *tritici* and 30° to 40° as that for *chinensis;* while Bruderlein (*3*) states that *maydis* makes its optimum growth at 39°. This is considerably higher than that obtained by the writers. No explanation for this apparent difference can be given.

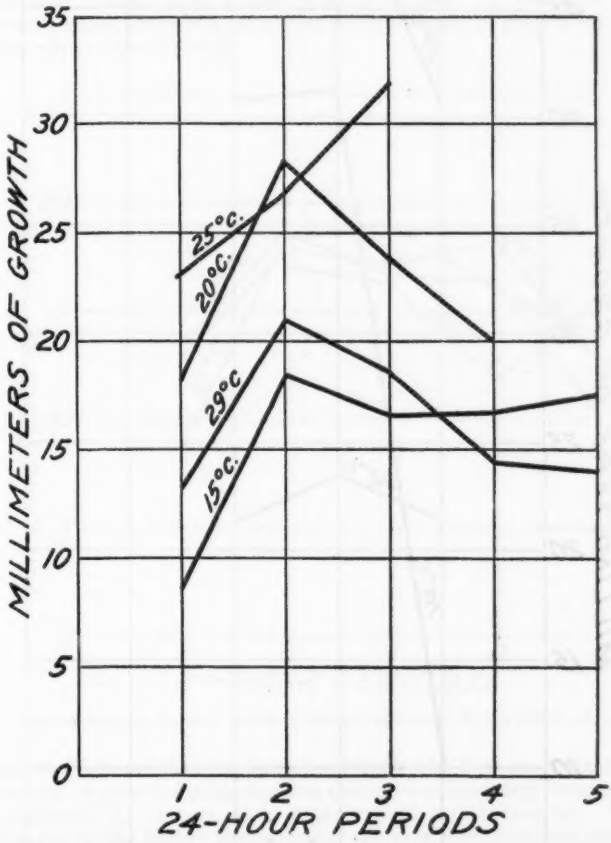

FIG. 14.—Graph showing the increase of growth of *Rhizopus nigricans* for consecutive 24-hour periods.

Graphs (fig. 13 to 23) were constructed to show the variation in the growth rate at different temperatures for successive 24-hour periods. The ordinates represent diameter increase in millimeters while the abscissas represent successive 24-hour periods after exposure to a given temperature. An inspection of these graphs shows that for most of the fungi the maximum rate of growth was attained during the second 24-hour period, the exceptions being *delemar* and *chinensis*, which reached their maximums one day later. The data for *nodosus*, although not alto-

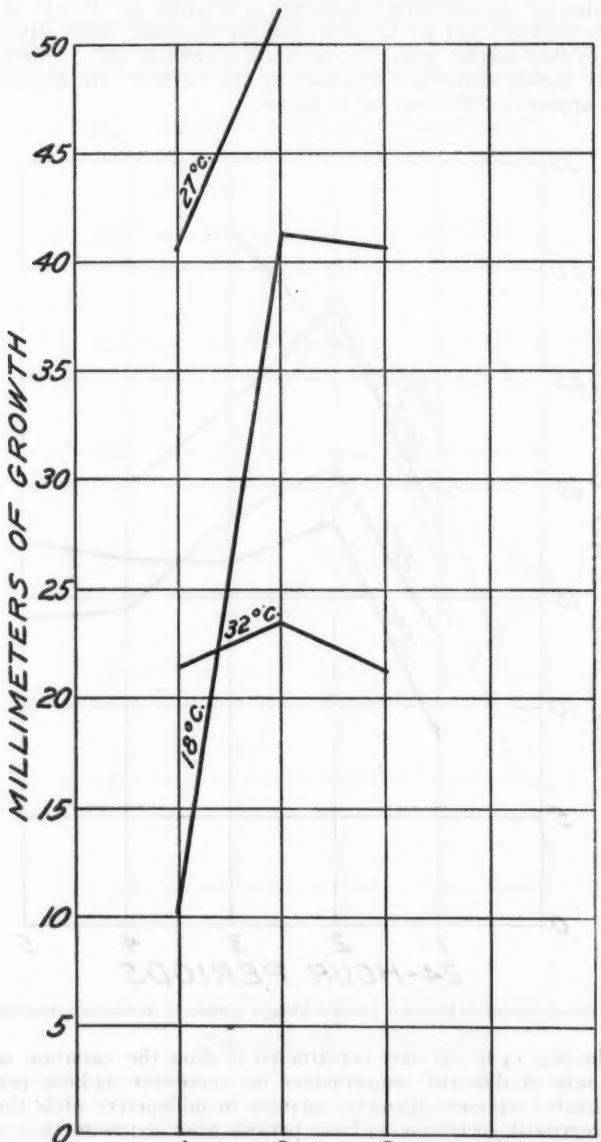

FIG. 15.—Graph showing the increase of growth of *Rhizopus reflexus* for consecutive 24-hour periods.

gether consistent, seem to indicate that it makes its most rapid growth some time after the second day. The growth rate varied with the temperature in most cases. In *arrhizus*, *tritici*, *maydis*, and *artocarpi* the obvious discrepancies are at the low temperatures, where the curve continues to rise after the second day. Certain of the fungi—*oryzae*, *artocarpi*, *arrhizus*, and *nigricans*—show a rather rapid decline in the rate of growth after the maximum is attained, while others, such as *microsporus*, *delemar*, and *reflexus*, show a more gradual decline. In *chinensis*, so far as the data show, there was no reduction in the rate of growth up to the time the plates were covered.

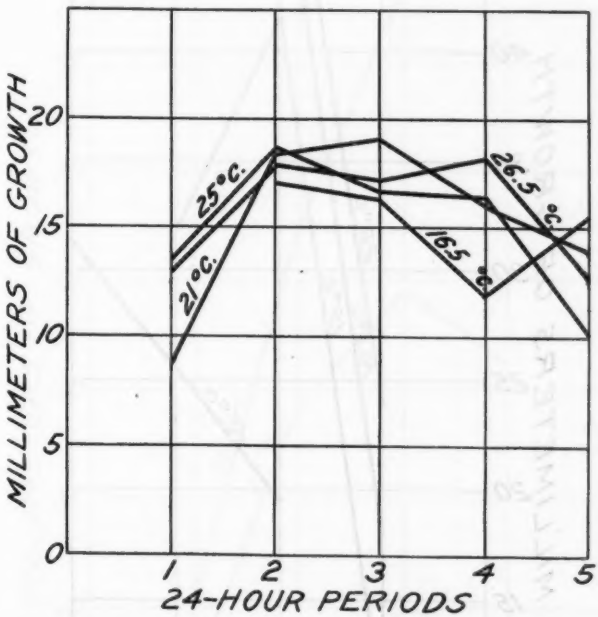

FIG. 16.—Graph showing the increase of growth of *Rhizopus microsporus* for consecutive 24-hour periods.

Fawcett (*6*) found that the fungi investigated by him made their greatest increase in growth during the first two days at the lower temperatures and continued to increase at a more gradual rate thereafter. Over a small range of the higher temperatures the rate first increased and then remained more or less constant till the end of the experiment, while at the highest temperatures a continuous decrease was noted from the first observational time. In general these results agree with those of the writers. Only in *oryzae*, *artocarpi*, and *tritici* at the highest temperatures (38°, 27.5°, and 42° C., respectively) do the graphs show a decrease, beginning with the first 24 hours. Nevertheless the fact that the maximum shifts, as explained above, indicates that such a condition would have been found to exist had the proper temperatures—for example, temperatures very near the maximum—been used.

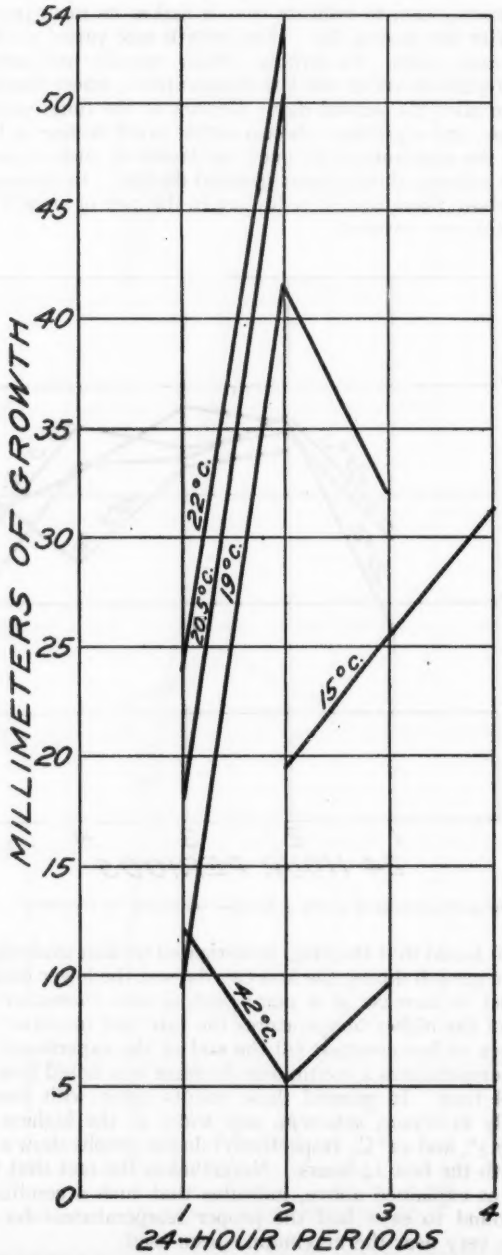

FIG. 17.—Graph showing the increase of growth of *Rhizopus tritici* for consecutive 24-hour periods.

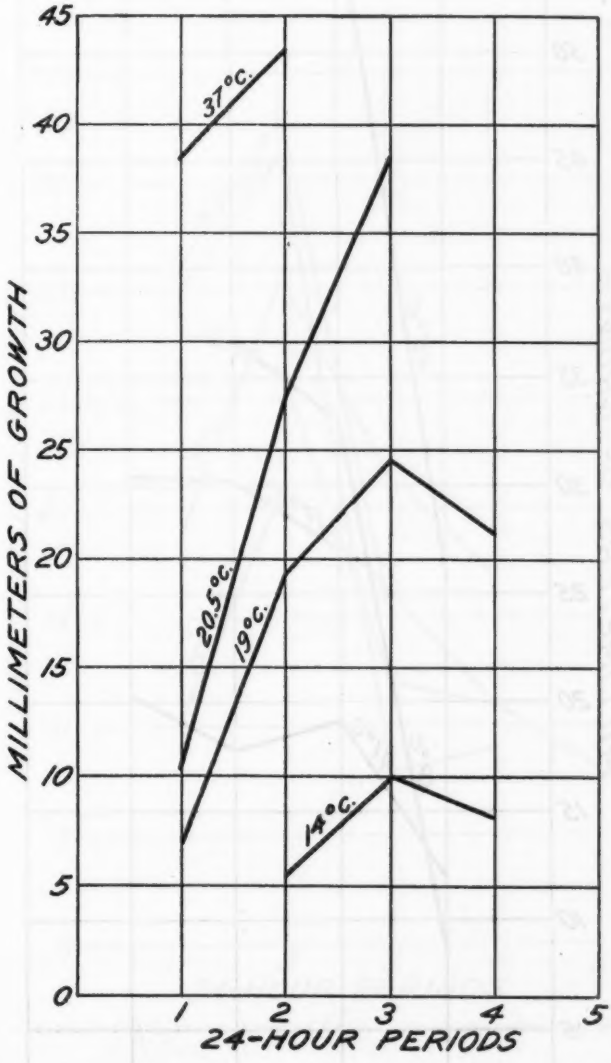

FIG. 18.—Graph showing the increase of growth of *Rhizopus delemar* for consecutive 24-hour periods.

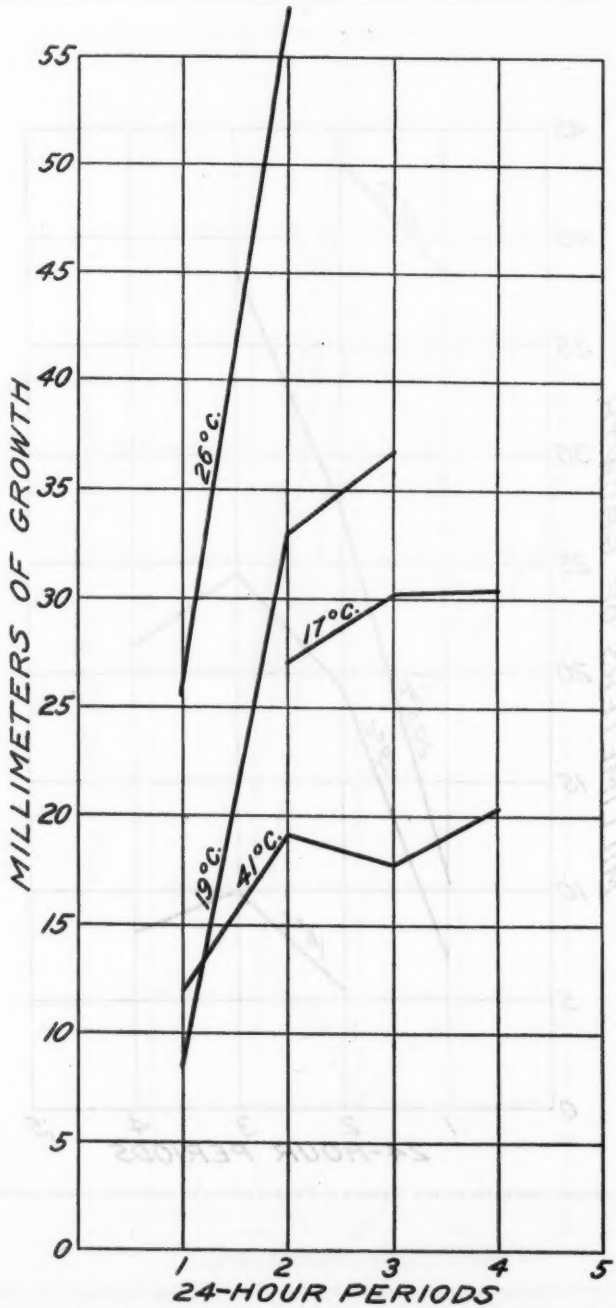

FIG. 19.—Graph showing the increase of growth of *Rhizopus nodosus* for consecutive 24-hour periods.

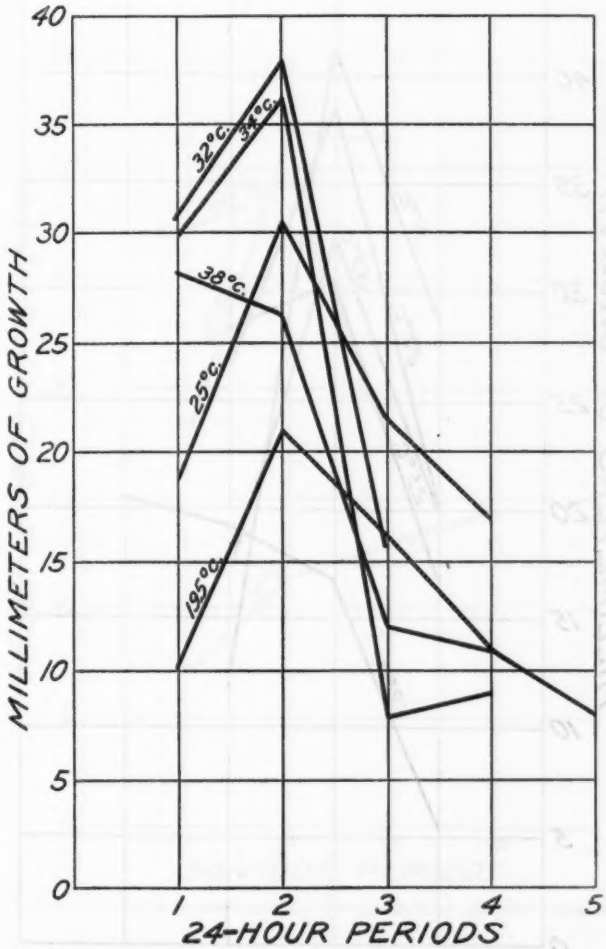

FIG. 20.—Graph showing the increase of growth of *Rhizopus oryzae* for consecutive 24-hour periods.

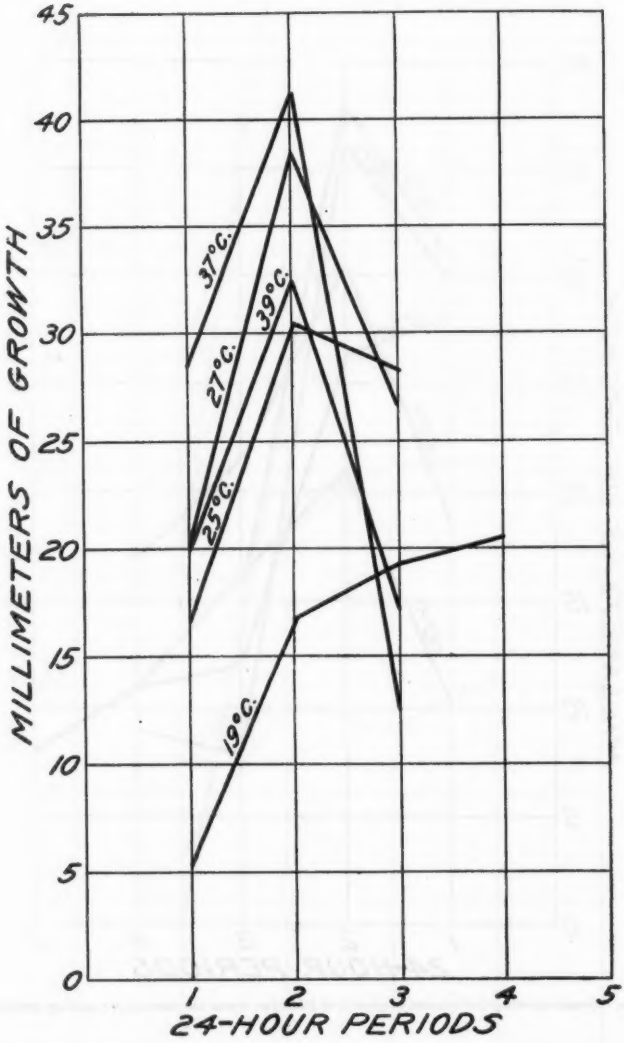

FIG. 21.—Graph showing the increase of growth of *Rhizopus arrhizus* for consecutive 24-hour periods.

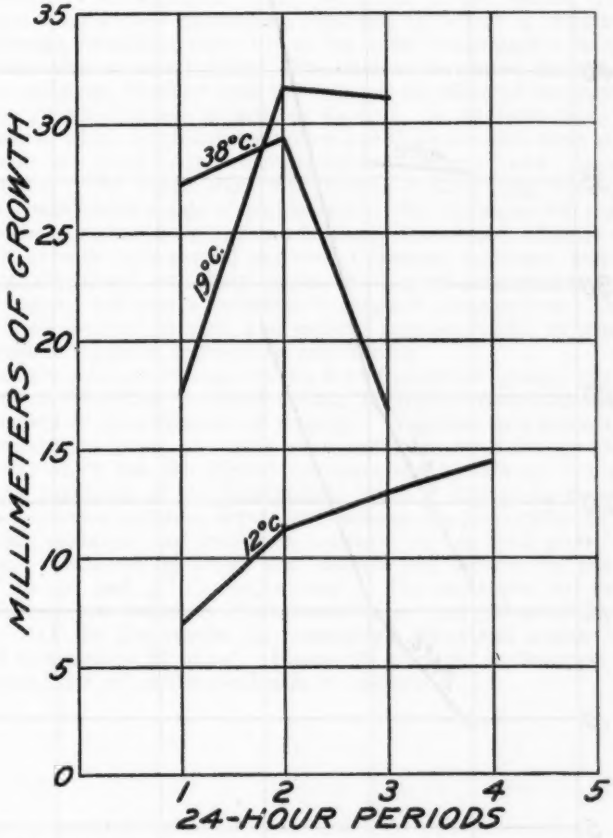

FIG. 22.—Graph showing the increase of growth of *Rhizopus maydis* for consecutive 24-hour periods.

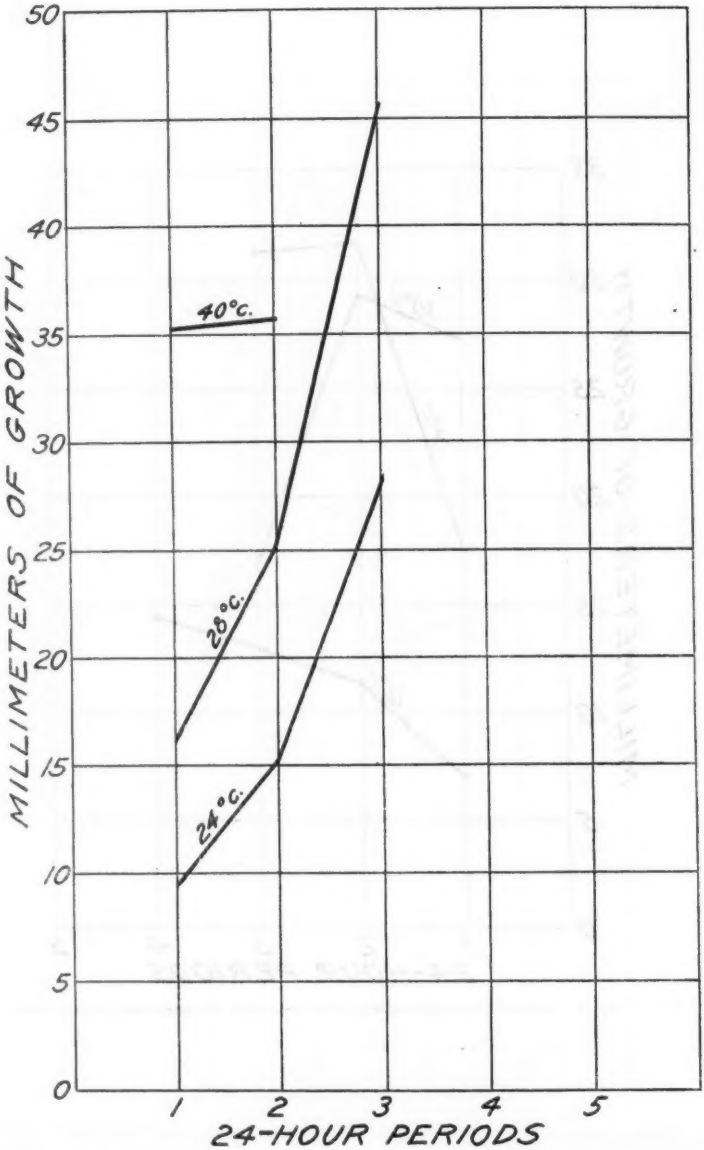

FIG. 23.—Graph showing the increase of growth of *Rhizopus chinensis* for consecutive 24-hour periods.

INFLUENCE OF TEMPERATURE ON FRUITING.

It has been repeatedly demonstrated by different investigators that the range of temperature at which fungi will sporulate is not coextensive with that at which they will grow. It has been proved that the various species of *Rhizopus* often cause considerable damage to vegetables and fruits. Although under suitable environmental conditions the causal organism can spread from host to host by means of their mycelium, their distribution is accomplished largely by means of the spores. It was therefore thought that information regarding the effect of temperature on sporangia formation, especially at the lower temperatures, might be of considerable practical value. The data accumulated during these studies are given, together with the data on the effect of temperatures on spore germination and growth, in Table I. In all cases those temperatures at which no growth took place and those at which some growth was made are listed in the columns headed "absent" and "present," respectively. The results show that fruiting of these fungi takes place over a considerable range of temperature. The optimum for some of the species is rather sharp and can be easily determined, while in other cases it extends over several degrees. *Chinensis*, *nigricans*, and *artocarpi*, which showed very sharp optimums for spore germination, fruited about equally well over a considerable range of temperatures. On the other hand, *arrhizus*, *reflexus*, and *nodosus* respond readily to temperature, both in regard to germination and fruiting.

Although *artocarpi* belongs to the low-temperature group, when its optimum is considered it stands among the highest-temperature forms with respect to its minimum for fruiting. *Nigricans* and *reflexus*, two low-temperature forms, also have a low minimum for fruiting; whereas *chinensis*, which has the highest optimum and maximum, has about the same minimum as the intermediate forms. The group consisting of *tritici*, *delemar*, *arrhizus*, *oryzae*, and *nodosus* has practically the same minimum, optimum, and maximum temperatures for both growth and fruiting. Hanzawa (*9*) found that *delemar* and *nodosus* did not fruit at 37° to 42° and 38° C., respectively. The maximum for *arrhizus* and *chinensis* was found by Hanzawa to be 36° and 38° to 42°, respectively. Of the five species just mentioned, *tritici* and *nodosus* alone formed sporangia at 8° to 10°. Hagem (*8*) gives 38° as the upper limit for *nodosus* and 36° as the maximum for *arrhizus*.

TABLE I.—*Minimum, optimum, and maximum temperature in degrees centigrade for spore germination, mycelial growth, and fruiting for 11 species of Rhizopus*

Species.	Spore germination.				
	Minimum.		Optimum.	Maximum.	
	Absent.	Present.		Present.	Absent.
artocarpi		1.5	26 to 29	33.5	34.5
nigricans		1.5	26 to 28	33	34
reflexus		1.5	30 to 32	36.6	38
microsporus		1.5	26 to 28	33	34
tritici		1.5	36 to 38	44	45.5
delemar	7	8.7	36 to 38	44	45.5
nodosus		1.5	36 to 38	44	45.5
oryzae	7	9	36 to 38	44	45.5
arrhizus		1.5	36 to 38	43.6	45.5
maydis					
chinensis	8.5	10	43 to 45	51	52

Species.	Mycelial growth.				
	Minimum.		Optimum.	Maximum.	
	Absent.	Present.		Present.	Absent.
artocarpi	7	9	26 to 28	32	33
nigricans	1.5	6.5	23 to 26	30.7	31.5
reflexus		1.5	26 to 28	33	34
microsporus		1.5	25 to 27	30	32
tritici	1.5	6.5	33 to 35	42	45.5
delemar	7.5	9.8	32 to 34	41	45.5
nodosus	1.5	6.5	33 to 35	41.5	45.5
oryzae	7.5	11	31 to 34	42	45.5
arrhizus	1.5	7.4	32.5 to 35.5	40.9	44.9
maydis	1.5	7.4	30.5 to 32.5	40	44.5
chinensis	7.5	10.4	37.5 to 40.5	49	51

Species.	Fruiting.				
	Minimum.		Optimum.	Maximum.	
	Absent.	Present.		Present.	Absent.
artocarpi	12.5	17	22 to 27	30	32
nigricans	7	10	23 to 28	30	32
reflexus	7.6	10.6	26 to 28	31	32.5
microsporus	[1]	[1]	[1]	[1]	[1]
tritici	12	16.5	32 to 34	40	45
delemar	12	15	32 to 34	38	40
nodosus	12.5	17	32 to 35	37	38.5
oryzae	12	15	32 to 36	37	40
arrhizus	11	15	32 to 34	37	40
maydis					
chinensis	12	16	35 to 40	45	50

[1] Not tested.

CERTAIN ENVIRONMENTAL FACTORS INFLUENCING GERMINATION AND GROWTH

TEMPERATURE AT WHICH THE SPORES ARE PRODUCED

Wiesner (*22*) found that the temperature at which the mycelium of *Penicillium glaucum* Link developed influenced the time required for the germination of the spores. For example, he showed that spores which matured at 14° C. germinated more quickly at 3° than those which had developed at 3°, while the spores that were produced at 22° germinated more slowly than those produced at 14°. The influence which the growing temperature of *Rhizopus nigricans* has on the germination of its spores has been studied to some extent by the writers. Stock cultures were grown for 7 days on Irish potato agar in Erlenmeyer flasks at 16°, 24°, and 27°. Spore suspensions were prepared in portions of the same solution. The hanging drops were prepared as previously described and incubated at 26°. The spores grown at 16°, 24°, and 27° formed germ tubes as long as the spores in 2 hours, in 2 hours and 5 minutes, and in 2 hours and 25 minutes, respectively. Similar tests were made with *delemar* with the following results: Spores produced at 20.5°, 27.5°, 31.5°, and 36.5° germinated at 36.5° after 3 hours and 45 minutes, 3 hours and 50 minutes, 4 hours and 45 minutes, and 7 hours + (no germination within 7 hours but some later), respectively. Tests with some of the other species showed a similar tendency—that is, spores which were produced at the lower temperatures germinated more quickly than those from cultures grown at higher temperatures. It was thought that possibly the difference in the rate of germination of the spores produced at different temperatures was due to a difference in age. The physiological activities of the organism are admittedly more rapid at the higher temperatures, the spores therefore being produced more quickly and probably aging more rapidly. In order to determine whether the difference in the rate of germination of spores produced at different temperatures might have been due to the age of the spores in the foregoing experiments, another test was made in the following manner: Fifteen 100-cc. Erlenmeyer flasks, each containing 30 cc. of Irish potato agar, were inoculated with a loop of a suspension of *nigricans* spores. Five of these flasks were then held at each of the following temperatures: 10°, 20°, and 26°. The time required for the development of the sporangia was noted in each case, and germination tests were made from time to time. The tests were conducted at 25.5°. The spores from cultures which had been fruiting for 3, 5, 8, and 12 days at 20° and 26° germinated in 3¼ hours. One flask held at 26° became contaminated and was discarded. The final test for the spores produced at 20° was made after 20 days, and the time required for germination was the same as when the other tests had been made. The spores produced at 10° when 3, 5, and 15 days old required only 2¾ hours for germination to start. The other two cultures failed to fruit. These experiments show first that spores produced at the lowest temperature (10°) germinated 30 minutes earlier than those formed at 20° and 26°, and second that the age of the spores, at least up to 20 days, did not influence the time necessary for the beginning of germination. It is quite evident from these results that it is important to grow the stock cultures used in comparative experiments at the same temperature.

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Experiments were next conducted to determine whether the influence of temperature on the germination of the spores is reflected in the rate of mycelial growth. Irish potato agar contained in 5 Petri dishes was inoculated with a loop of a spore suspension from cultures of *tritici* and *delemar* grown at 20.5°, 27.5°, 31.5°, and 36.5° C. The plates were held at 34° to 35°. At the end of 24 and 48 hours the growth of *tritici* had reached a diameter of 50.6, 50.8, 48, and 45.8 mm. and 84, 92, 82, and 79.5 mm., respectively. The growth of *delemar* measured at the end of 24 and 48 hours 46, 42, 40.5, and 34.6 mm. and 79.5, 81.4, 77, and 78.8 mm., respectively. In general *tritici* showed a slight decline in the rate of growth when the spores were grown at the two highest temperatures. *Delemar* showed a more marked difference at the end of the first 24 hours, there being a gradual decrease from 46 to 34.6 mm.; but this was largely overcome during the next 24 hours, as shown by the fact that there was less than 1 mm. difference between the two extreme temperatures. The temperature at which the spores are produced undoubtedly influences their rate of germination and the early period of the growth of the mycelium developed therefrom. The evidence seems to point to the fact that different species react differently in this respect, some being much more sensitive to small changes in temperature than others.

CULTURE MEDIA

Tests were also made to determine to what extent, if any, different media would affect the rate of germination of *nigricans* spores. The media used were sweet potato decoction, beef bouillon, distilled water, string bean agar, Irish potato agar, and a synthetic agar. The media were placed on cover slips, spores from a single culture were sifted on, and the slips were then inverted over glass rings on slides in the usual manner and incubated at 26° C. The time necessary for germination in each case was as follows: Sweet potato decoction, 2 hours; beef bouillon, 2 hours and 25 minutes; distilled water, 3½ hours; string bean agar, 2 hours and 10 minutes; Irish potato agar, 2 hours and 20 minutes; and synthetic agar, 3 hours. Sweet potato decoction proved the best solution tried, and string bean the best agar. This experiment was duplicated on different days, using spores from a different culture with very similar results. It seems clear that Rhizopus spores require something more than water for good germination, since in distilled water a much smaller percentage of the spores germinated, and the germ tubes produced were considerably more slender than those supplied with nutrients. Tap water was found by comparative tests to be a less favorable medium for the germination of these spores than distilled water.

In order to test the effect of the substrate upon the rate of mycelial growth, plates were prepared in the usual manner, using string bean and Irish potato agars. The plates were inoculated with a platinum loop of the same suspension of *nigricans* spores in sterile distilled water. Five plates of each agar were used at each temperature. Measurements of the diameters of the mycelial disks were made in the usual way, and an average taken of the growth on each medium at the different temperatures. The results are given in Table II.

TABLE II.—*Comparative rate of growth of Rhizopus nigricans on string bean and Irish potato agars at different temperatures*

Temperature.	24 hours.		48 hours.	
	String bean agar.	Irish potato agar.	String bean agar.	Irish potato agar.
°C.	*Mm.*	*Mm.*	*Mm.*	*Mm.*
33	0	0	0	0
25.7	41.4	23.8	92	46.6
23.3	40.4	22	92	42.2
19.4	22.4	13.8	73.8	39
12.9	7.4	6.2	48	24
11.9	0	0	9	7.8
10.7	0	0	0	0

The data show that for the most part *nigricans* grows nearly twice as fast on string bean agar as on Irish potato agar. The plates of bean agar at 25.7° C. after 48 hours were completely covered, and it was evident that the diameter of the growth would have been somewhat greater had it not come in contact with the edge of the dish. The plates at 23.3° were just covered. The difference in the amount of growth on the two agars at 11.9° was very slight but yet apparent. The cardinal temperatures on the two media appear from the result of these experiments to be about the same. However, to determine this point with certainty, much closer temperatures than those used by the writers would have to be employed.

DEXTROSE

While studying the effect of different culture media upon growth it was observed that *nigricans* grew at 30° C. on a synthetic agar but failed to do so on the other media used.

It was thought that perhaps the differences in the acidity of the media might account for this shifting of the maximum temperature. Hence, an experiment was prepared in which Irish potato and beef agars made up to the same H-ion concentration as the synthetic agar, were used, together with synthetic agar as a control. The fungus, however, failed to grow on either of the modified agars at 30° C., but did grow on the synthetic agar. This experiment demonstrated that the H-ion concentration of the media was not the cause of the shifting of the maximum temperature of this fungus.

The synthetic agar differed from the other agars in sugar content as well as acidity, and for this reason tests were made to determine the effect of the addition of different quantities of dextrose to the media. Irish potato and beef agars were prepared to contain roughly 1, 5, 10, 15, and 20 per cent and carrot agar 1, 5, and 10 per cent dextrose. Ten plates were prepared in the usual way with these as well as with unmodified string beans, corn meal, and sweet potato agars. Plates of synthetic, Irish potato, beef, and carrot agars were prepared in the same manner and held as controls. All the plates were held at 30° C. for 48 hours, when the diameter of the growth was measured. This experiment was repeated several times. The average figures for all tests are presented

in Table III. In the cases where no growth is recorded the spores usually had germinated and formed short germ tubes but never formed a spot which spread appreciably beyond the area covered by the drop of spore suspension. Exposure for 48 hours to a temperature at which no growth took place was sufficient to kill the fungus, as was shown by the fact that it failed to make a further growth when held for 24 hours at a temperature suitable for its development.

TABLE III.—*Comparison of the growth made by Rhizopus nigricans at 30° C. on different agars with and without dextrose added*

Agar used.	Not modified.	With addition of dextrose.				
		1 per cent.	5 per cent.	10 per cent.	15 per cent.	20 per cent.
			Mm.	*Mm.*	*Mm.*	*Mm.*
Irish potato	No growth	No growth.	7	49	59	57
Beef	do	do	No growth.	30	52	54
Carrot	do	do	do	23		
String bean	do					
Corn meal	do					
Sweet potato	do					
Synthetic	[1] 47					

[1] Contained 20 per cent dextrose.

The table shows that the strain of *nigricans* used (4652) did not grow at 30° C. on any of the unmodified agars tried except synthetic agar, which contained 20 per cent dextrose as originally prepared. Upon the addition of 10 per cent or more of dextrose to Irish potato, beef, or carrot agar growth did take place at this temperature. The addition of more than 15 per cent of sugar seemed to have little effect.

After it was determined that the maximum temperature for growth was raised by the addition of dextrose to the medium, experiments were conducted to learn the extent of this change. A loop of the same spore suspension was placed in the center of each of 20 Petri dishes, one-half of which contained unmodified Irish potato agar and the other half some of the same agar to which 20 per cent dextrose was added. The plates were then held at 31° C. Observations after 48 hours showed that the spores had not germinated on the unmodified agar while an average growth about 5 mm. in diameter had been made on the agar with 20 per cent dextrose added. A slight additional growth was made after 48 hours longer on the agar with sugar added. The results of these experiments seem to justify the conclusion that the maximum temperature for the growth of this organism on Irish potato agar is raised from 1° to 1.5° C. by the addition of 20 per cent dextrose to the medium.

That the presence of dextrose in the medium influences the minimum temperature was demonstrated by placing a loop of spore suspension in the center of each of 20 plates of Irish potato agar to one-half of which 20 per cent dextrose was added. The plates were then placed at a temperature of from 0.8° to 2° C. The spores had not germinated at the end of 17 days on the unmodified agar. On the other hand, germ

tubes of from three to five times as long as the diameter of the spores had formed on the agar containing 20 per cent dextrose. The differences on the two agars seem to indicate that the presence of 20 per cent dextrose in Irish potato agar lowers slightly the minimum temperature for germination.

To determine whether the optimum would be changed by the addition of dextrose, plates of each of the agars were prepared and placed at temperatures of 23, 26.5, 27.8, 28.7, and 30° C. Measurements of the diameters of the mycelial growths after two days at 23, 26.5, 27.8, 28.7, and 30° on the unmodified agar were 42, 65.6, 61, 51.8, and 0 mm., and on agar with 20 per cent dextrose added 66.4, 87.8, 92, 92, and 83.2 mm., respectively. These figures show that the optimum for growth on the unmodified agar was 26.5°, while that on the agar containing 20 per cent dextrose probably lay between 27.8 and 28.7°. The measurements of the diameters of the growths at 26.5, 27.8, and 28.7° after 24 hours were 26, 36, and 34 mm., respectively, showing that the optimum was nearer 27.8° than 28.7° but nearer the latter than 26.5°, and was raised 1.5° to 2°.

These experiments show rather conclusively that the presence of dextrose in Irish potato agar shifts the cardinal temperatures of this strain of *Rhizopus nigricans*. Thiele (*21*) also found that the addition of dextrose to glycerine and formic acid changed the maximum temperatures for the growth of one species of both Penicillium and Aspergillus. He observed that dextrose added to glycerine and to formic acid caused a shifting of the maximum temperatures for the growth of Penicillium of 5° and 4° C., respectively. The temperature maximums for the germination of Aspergillus spores and for mycelial growth were raised about 2° and 3°, respectively, by the addition of different amounts of dextrose. On the other hand the maximum for total growth was raised about 2° when growing on glycerine and lowered 3° on the higher concentrations of formic acid. He concluded that the nutritive value of a substance is correlative to a certain extent with the temperature. Similarly, Bruderlein (*3*) found the growth of *R. maydis* to be retarded on potato, almost arrested on carrot, and prohibited entirely on agar at 42°. Peltier (*15*) showed that the minimum temperature for the growth of *Psuedomonas citri* Hasse when grown in beef bouillon and on soluble starch agar is slightly higher than on cooked potato cylinders, its maximum being slightly higher in the former than on the two latter media. Likewise, Goss (7) found that *Fusarium trichothecioides* Wollenw. made its best growth on a synthetic solution at 25° and no growth at 5°. On the other hand, the optimum lay between 15° and 20°, and a weighable growth was made at 5° when this fungus grew on Link's potato extract.

Although it has been demonstrated by the writers and others that the cardinal temperatures of some fungi vary with the nature of the substrate, the principles underlying this phenomenon have never been explained. Thiele (*21*) attributes the shifting of the maximum temperature of the fungi with which he worked to the different nutritive value of the substrate at different temperatures. It may be in the case of the writers' experiments that the dextrose when present in considerable concentration (10 per cent or over) was more available and hence supported growth at a higher temperature than when it was present only in small amounts. It is a well-known fact that increasing the molecular concentration of a solution raises its boiling point and lowers its freezing point. It is not known whether or not the increased concentration of the dextrose in the substrate causes a sufficient increase in the molecular

concentration of the cell sap, thereby protecting the protoplasm of the cell against coagulation by the heat to account for the raising of the temperature maximum 1° to 1.5° C. The high concentration of sugar in the substrate may also act to lower the water content of the cells of the fungus and thereby increase the concentration of the cell sap which would tend to raise its maximum and lower its minimum. However, the true explanation of this phenomenon must await the accumulation of more experimental data.

DISCUSSION

It is apparent from the foregoing curves and tables that the cardinal temperatures for spore germination, growth, and fruiting of the fungi studied vary somewhat. In general the spores will germinate at a temperature too low for mycelial growth. A higher temperature is required for fruiting than for growth. The same thing holds true for the optimum temperatures, although not to the same extent. The optimum for germination is always higher than for growth and fruiting, while in most cases the optimum for fruiting is about the same as that for growth. The slight differences shown in Table I may be due to the fact that these figures give the optimum temperatures tried rather than the true optimums. The latter probably does not differ from the former more than a plus or minus 1 or 2° C., since the temperature of the incubators usually differed only from 2 to 5°, the maximum differences being between the extreme temperatures. The optimum temperature for fruiting is often not so well defined as that for growth, and the latter less so than for spore germination.

In each case there is a gradual gradation in the maximum from that at which the spores will germinate to that at which fruiting will take place, the maximum for growth being about midway between that for germination and that for fruiting. This fact may be explained in part at least in two ways; first, the spores germinate quickly, probably before the heat acts injuriously; and second, the spores are probably less sensitive to heat than are the germ tubes. Spores which before germination are uninjured by the heat may be readily killed after they germinate. At a slightly lower temperature growth becomes evident but ceases after a time, and the mycelium is killed before sporangia are formed.

The time factor is of greater importance at the minimum than at any other temperature. At low temperatures, germination is greatly retarded, the growth being often so sparse that no measurable felt is produced within the time limit of these experiments.

The genus Rhizopus includes some of the best known, most widely distributed and most destructive of the fungi. *Nigricans* is perhaps the most destructive, since it attacks a wide range of hosts under widely different conditions, being especially destructive to tomatoes, strawberries, and sweet potatoes. However, this species is somewhat limited in its field of destruction by temperature. For example, in these experiments its spores were killed by a comparatively short exposure at a temperature of 34° to 35° C., and it made a very slow growth at 6.5° (35 mm. in 30 days) and no appreciable growth at 1.5° after 30 days. Although some variation in its response to temperature on different substrates may be expected, there is little doubt that decay of fruits and vegetables by this organism can be prevented or retarded by proper cold storage. A temperature as high as 7° or thereabouts will prevent fruiting for several days and hence retard the spread of this fungus. It

must be kept in mind, however, that other species may be destructive at temperatures which are not favorable for the development of *nigricans*, although at the present time little is known regarding the distribution and destructiveness of the other species under natural conditions in the United States.

SUMMARY

(1) The effect of temperatures on the spore germination, mycelial growth, and fruiting of 11 species of Rhizopus has been studied, and the results are presented in this paper.

(2) These 11 species fall into three groups as regards their response to temperature. *Chinensis* has a temperature maximum and optimum several degrees higher than any of the other species and hence can be easily separated from them; *nigricans*, *microsporus*, *reflexus*, and *artocarpi* make up a group having a low optimum and maximum; while the 6 remaining species, namely, *tritici*, *nodosus*, *delemar*, *oryzae*, *arrhizus*, and *maydis* constitute an intermediate group.

(3) In general the spores will germinate at a temperature above the maximum for continued growth.

(4) The optimum for germination for all the species is higher than for growth and fruiting, while in most cases the optimum for fruiting is about the same as for growth.

(5) The temperature at which the spores are produced influences to some extent their rate of germination and the early period of the growth of the mycelium developed therefrom. Spores of *nigricans* produced at 10° C. germinated in 30 minutes less time than those formed at 20° and 26°. Spores of this species from different cultures grown under like conditions germinated equally well, regardless of age, up to 20 days.

(6) Spores of *nigricans* germinate in a considerably shorter time in a nutrient solution than in water. Sweet potato decoction proved to be the best liquid and string bean agar the best solid medium tried. In the comparative tests made this fungus grew nearly twice as fast on string bean agar as on Irish potato agar.

(7) The presence of 20 per cent dextrose in Irish potato agar changed the cardinal temperatures of the strain of *nigricans* studied from 1 to 2° C.

(8) *Nigricans*, which seems to be the most destructive member of this genus, under natural conditions is somewhat limited in its field of destruction by temperature. The spores were invariably killed at 35° C., and growth was very sparse and slow at 6.5°. At 1.5° no appreciable growth was made on Irish potato agar in 30 days.

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NUTRITION OF PLANTS CONSIDERED AS AN ELECTRICAL PHENOMENON [1]

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INTRODUCTION

When wheat seeds are placed in a suitable nutrient solution and germination begins, the demand of the embryo and tiny seedling for food is first upon the stored up material in the seed. As the plumule and radicle develop, however, absorption of food from the nutrient solution begins and gradually increases with increased growth. During the germination process there is first an exudation of plant food, particularly phosphorus and potassium, from the seed, with little or no absorption from the nutrient solution, but, when the plumule has reached a length of 2 or 3 cm. and has broken through its sheath, a distinct demand for food begins to be manifested in the tissues.

It is of much importance in fertilizer investigation to note that this demand of the plant for nutrient material may be measured by its pull upon the nutrient solution, and it is equally important from a physiological standpoint to note that this demand may be modified in certain ways and may even be augmented or built up by withholding any of the food elements. There is a "residual effect" that is very pronounced in plants; the desire for food when not available is carried over a long period of time and it seems to be cumulative. In this respect a plant seems to behave very much like an animal organism, the demand for food increasing as a fast continues. There is another very pronounced phenomenon that might well be termed a "time factor." A plant will not necessarily absorb twice as much nutrient material in two hours as it absorbs in one. It seems to possess the power to prepare reserves—that is, to form compounds in one part of its system which may be translocated to another part when needed.

It seems that this "demand" for food must be taken into account in all studies of absorption.

DEMAND OF WHEAT PLANTS IN THE PRESENCE OF A CONTINUOUS SUPPLY OF PLANT FOOD

About a dozen culture pans were prepared, each pan holding 2,500 cc. of nutrient solution and containing about 500 plants. The plants were grown by sprinkling seed upon floating perforated aluminum disks, and the nutrient solutions, containing 125 parts per million each of nitrogen (N), potash (K_2O), and phosphoric acid (P_2O_5) as sodium nitrate, potassium chlorid, and sodium phosphate, were changed daily. There was thus an abundance of plant food always at the disposal of the plant, and this was determined by measurements made from time to time. A

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concentration of 125 parts per million is just about the concentration producing the best growth under these conditions, as shown by other work; and as will be seen later, there was very little likelihood of the plant absorbing very much more plant food than was actually needed for its development.

Beginning with the fifth day after the seeds were placed in the solution, on every third day enough plants for an analysis were drawn from the pans, care being taken to draw some plants from each pan to equalize variations in the cultures as much as possible. When one of the culture pans fell below the normal it was discarded. In this way a representative set of plants could be withdrawn each time. The analyses of the plants, together with that of the original seeds, are shown in Table I, the results being expressed on the basis of 100 plants.

TABLE I.—*Analyses of 100 wheat seedlings, grown in nutrient solutions, at different stages of growth*

No.	Stage of growth.	Dry weight.	N	K_2O	P_2O_5
		Gm.	*Gm.*	*Gm.*	*Gm.*
1	Original seeds	2.14	0.0490	0.0295	0.0210
2	5 days old	1.89	.0565	.0272	.0184
3	8 days old	2.05	.0742	.0489	.0250
4	11 days old	1.82	.0812	.0869	.0308
5	14 days old	1.95	.0924	.1203	.0336
6	17 days old	2.30	.1160	.1311	.0570
7	20 days old	3.04	.1410	.1466	.0740
8	23 days old	3.50	.1946	.1610	.1020
9	26 days old	4.64	.2110	.2018	.1220

It will be noticed that there is a falling off in dry weight, in potash, and in phosphoric acid, but not in nitrogen, up to the fifth day. The loss in weight is, of course, due largely to the decomposition of starch and sugar of the seeds and to the evolution of carbon dioxid. This loss often amounts to as much as 40 per cent of the dry weight before enough plant food and carbon dioxid are absorbed to balance the loss. Many experimenters with wheat seedlings, when using dry weight as a criterion, fail to realize the fact that in the first stage of growth they are dealing with a diminishing quantity. The loss of potash and phosphoric acid always takes place at the beginning of germination, due to the exudation of these plant foods from the seed. It might be added that these very salts that are exuded from the seeds are absorbed by the seedling in a few days or as soon as the radicle has become sufficiently developed. There is usually little exudation of nitrogen for the reason that the nitrogen of the seed exists in organic combinations, protein, and is not readily dissolved out by water.

These results when plotted are represented by figure 1.

By cutting the curves through any particular date it seems possible to determine the relative demand of the plant at that age for the three important plant foods. It is also evident that the relative demand for food changes very rapidly as the plant develops. Beginning about the fifth day, when the plants begin to feed, the curve for potash rises very rapidly. The little seedling awakes to life with a ravenous appetite for potash, out of proportion to other plant foods. When the seeds are moistened and warmed preparatory for germination, the potash which

is stored up in the seed, and which is fairly evenly distributed through it, begins to move very rapidly toward the end containing the germ or embryo. In the germination, when the embryo has broken through the bran coating and is just large enough to get hold of with the thumb and finger nails, it contains about 50 per cent of the total potash of the seed. The seed does not contain enough potash to satisfy the demand of the little seedling, so it begins early to feed heavily on the nutrient of the solution. Probably, in the natural course of life, the very first food absorbed by the little seedling is potassium. The absorption of nitrogen is steady and comparatively uniform, so after 18 or 20 days the curves for potash and nitrogen cross each other. The absorption of phosphoric acid is regular also.

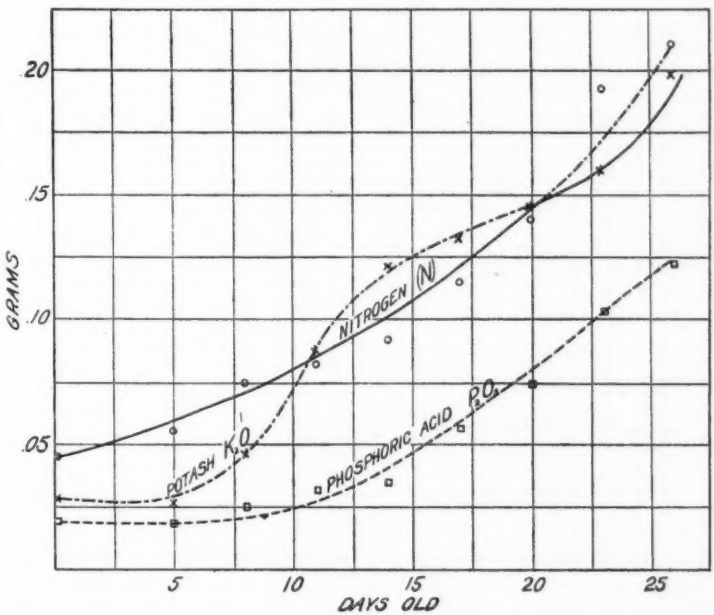

FIG. 1.—Graph illustrating the absorption of plant foods from nutrient solutions by wheat seedlings at different stages of growth.

Without submitting all the data, the curves for a duplicate determination, made about one month later, are given in order to bring out the crossing of the curves of nitrogen and potash (fig. 2).

The data presented give an idea of the nutrition of a plant when feeding full time and under favorable conditions. But a plant in nature does not necessarily have ideal conditions in which to grow. A low moisture content of the soil may temporarily put a stop to nutrition or the fluctuation of plant food, particularly nitrates, in the soil solution may also slow down, or even stop, absorption. It is, therefore, important to know what percentage of the time is actually necessary for absorption and whether a plant can absorb enough plant food in one period of time to last it over another.

DEMAND OF WHEAT PLANTS GROWN IN NUTRIENT SOLUTIONS FOR FRACTIONAL PARTS OF A DAY

Seedlings were placed in culture pans containing a full nutrient solution of a concentration of 125 parts per million each of nitrogen, potash, and phosphoric acid, as before described, and allowed to feed for fractional parts of the day. At the end of the period allotted to each lot to remain in the nutrient solution, the disks with the seedlings were taken up and washed off and placed in distilled water for the remainder of the day. This process was repeated daily for 10 days, and the plants then analyzed for nitrogen, potash, and phosphoric acid. In Table II is

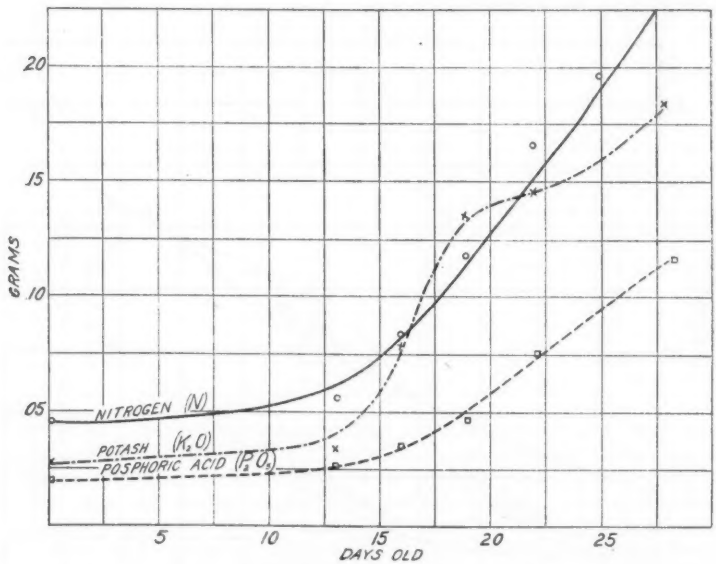

FIG. 2.—Graph illustrating the absorption of plant foods from nutrient solutions by wheat seedlings at different stages of growth.

shown the number of hours each day the plant remained in the nutrient solution and the quantities of plant food absorbed.

TABLE II.—*Analyses of 100 wheat seedlings feeding fractional parts of the day for 10 days*

No.	Hours per day in nutrient solution.	Dry weight	N	K_2O	P_2O_5
		Gm.	*Gm.*	*Gm.*	*Gm.*
1	Control 0	5.32	0.0770	0.0318	0.0420
2	1	5.15	.0882	.0838	.0560
3	2	5.60	.1022	.1047	.0750
4	4	5.20	.1120	.1366	.0840
5	6	5.80	.1540	.1862	.0960
6	8	5.30	.1680	.1940	.1040
7	12	5.73	.2072	.1858	.1060
8	24	6.28	.2352	.2475	.1190

It will be seen by referring to Table II and figure 3 that the absorption of plant food for 1 hour per day, and other fractional parts, is out of proportion to what might be expected if time alone governed absorption. For example, subtracting the amount of potash in the original seed, or in the control, from the analysis of the plants grown 1 hour a day in the nutrient solution, we get 0.0520 gm. of potash actually absorbed by the plants during that time. In the same way by subtracting the control from those grown 24 hours a day in the nutrient solution,

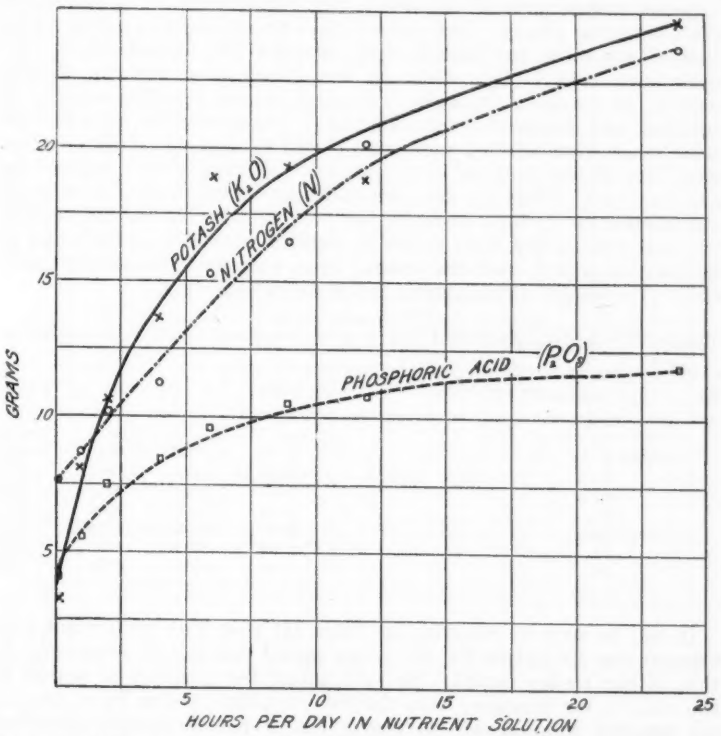

FIG. 3.—Graph illustrating the absorption of plant foods by wheat seedlings when grown for fractional parts of the day for 10 days in nutrient solutions.

we get only 0.2157 gm. of potash actually absorbed in 24 hours. If the rate of absorption of the plants kept in the nutrient solution 1 hour a day had been maintained in the plants kept in 24 hours a day, we would have had an absorption of 1.248 gm. instead of 0.2157 gm. In other words, we get over five times as much potash absorbed in the 1-hour periods as might be expected if time alone governed absorption. This phenomenon is true with other plant foods; the curves rise very abruptly from the start and flatten out as the time increases. The demand or desire for food seems to accumulate until the food becomes available; then an abnormal rate of absorption takes place. The figures, when plotted, are represented in figure 3.

The abrupt rise of the potash curve is noticeable, showing the demand for potash in the seedling was out of proportion to that for other plant foods. The cumulative demand, brought out in this experiment, seems to be true, not only for fractional parts of the day but for much longer periods.

DEMAND OF WHEAT PLANTS AFTER BEING HELD IN DISTILLED WATER FROM 2 TO 17 DAYS

Seven culture pans of soft wheat were germinated and grown in distilled water for 4 days. One pan was then transferred to a nutrient solution of 125 parts per million each, nitrogen (N), potash (K_2O), and phosphoric acid (P_2O_5), while all the others were kept in distilled water. At the end of 3 more days a second pan was placed in the nutrient solution, and this process continued every 3 days until six of the lots had been taken from distilled water and placed in nutrient solution. They were then allowed to grow for 2 more days, when they were taken down and analyzed. Thus the first pan had been feeding from a good nutrient solution for 17 days, while the last pan had been feeding only 2 days. The last pan having been grown in distilled water for 15 days had no increase in growth over the control when placed in the nutrient solution. The results of the analyses are shown in Table III.

TABLE III.—*Analyses of 100 wheat seedlings grown two or more days in nutrient solutions*

No.	Days in nutrient solutions.	Dry weight.	N	K_2O	P_2O_5
		Gm.	*Gm.*	*Gm.*	*Gm.*
1	Control 0	6.28	0.0840	0.0613	0.0400
2	2	6.60	.1410	.1154	.0587
3	5	6.52	.1904	.1685	.0750
4	8	7.12	.2268	.2090	.0813
5	11	6.80	.2674	.2350	.0973
6	14	6.80	.2604	.2361	.0908
7	17	7.00	.2912	.2580	.1440

It will be seen by referring to Table III that with each plant food element the absorption for the 2-day period was out of proportion to that of the 17-day period. By subtracting the quantity of potash in the control, for example, from that of the plants grown for 2 days in the nutrient solution, we get 0.0541 gm. of potash actually absorbed in 2 days. In like manner, by subtracting the control from the 17-day plants, we get only 0.1967 gm. potash actually absorbed in 17 days, when we should get 0.459 gm. of potash if time alone governed absorption. These results plot very well, showing that the demand is fairly regular and cumulative. This experiment was repeated, changing the plants in 2-day periods, with similar results.

If Tables IV and V, showing the rates of absorption of the different plant foods, are studied, other interesting relations are brought out. From Table IV it appears that the absorption of potash for the short period is much more pronounced than the absorption of other plant foods. On the other hand, when seedlings are left in the nutrient solution for a number of days (Table V), the absorption of potash and nitrogen shows a remarkable agreement in the rate. This is brought out in figures 4 and 5.

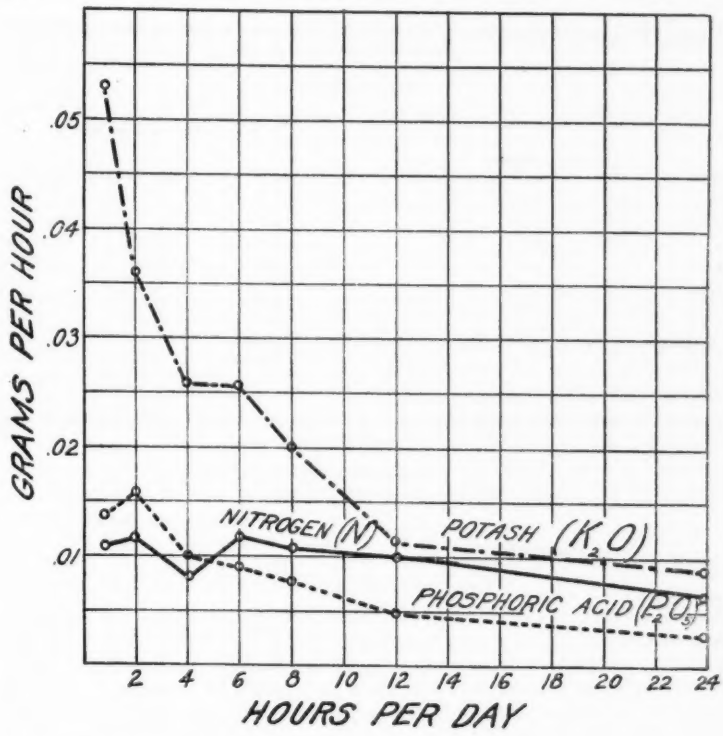

FIG. 4.—Graph illustrating the rate per hour of the absorption of plant foods by wheat seedlings when grown for fractional parts of the day in nutrient solutions.

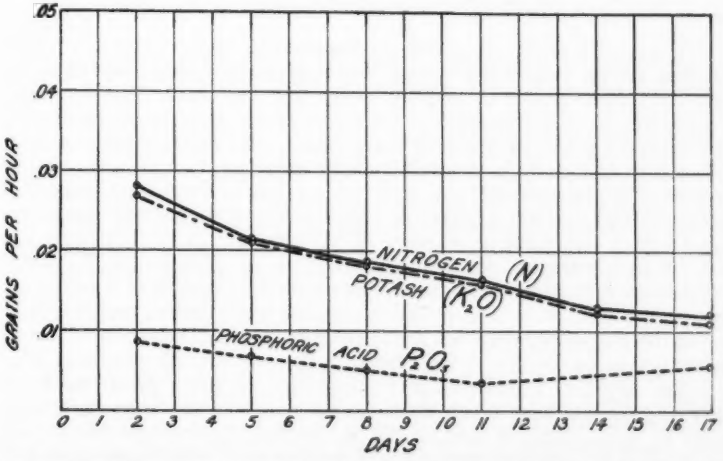

FIG. 5.—Graph illustrating the rate per day of the absorption of plant foods by wheat seedlings when grown for two or more days in nutrient solutions.

TABLE IV.—*Rate of absorption of plant foods per hour when wheat seedlings were feeding fractional parts of day*

[From Table II]

Hours per day.	Rate of absorption per hour.		
	N	K_2O	P_2O_5
	Gm.	*Gm.*	*Gm.*
1	0.00112	0.00520	0.00140
2	.00126	.00364	.00165
4	.00087	.00262	.00105
6	.00128	.00257	.00090
8	.00114	.00203	.00078
12	.00108	.00128	.00053
24	.00066	.00090	.00032

TABLE V.—*Rate of absorption of plant food per day when wheat seedlings were feeding for long periods*

[From Table III]

Days in solution.	Rate of absorption per day.		
	N	K_2O	P_2O_5
	Gm.	*Gm.*	*Gm.*
2	0.0285	0.02705	0.00935
5	.0213	.02144	.007
8	.01785	.01846	.00516
11	.01667	.01579	.00521
14	.0126	.01249	.00363
17	.01219	.01157	.00612

DEMAND OF WHEAT PLANTS GROWN FOR AN INITIAL PERIOD IN A FULL NUTRIENT SOLUTION AND SUBSEQUENTLY STARVED

The second of the two experiments described above was made in an effort to measure the demands for plant food that might be developed in plants that had been grown in distilled water and had never had any food supply except that contained in the seed. It was thought probable that other results might be obtained if the plants were first fed heavily, then allowed to fast, and the demand brought about by this fast measured in a second feeding period. A separate set was started, and when the plumules had reached a length of about 1 cm. all the culture pans were placed in a full nutrient solution of a concentration of 100 parts per million each nitrogen (N), potash (K_2O), and phosphoric acid (P_2O_5) and allowed to remain in this solution with frequent changes of solution for seven days. All were then placed in distilled water and allowed to stand seven days or more, when No. 4 was again placed in the nutrient solution. After one more day No. 3 was placed in the nutrient solution, and after another day No. 2 was put in the nutrient solution. After one more day all four sets were taken down for analysis. In this way, the plants were all given plenty of food for the first period, then allowed to fast and to develop an appetite during the second period, which was measured in the third period. This is shown in Table VI.

TABLE VI.—*Analyses of 100 wheat plants given an abundance of plant food in the first period, placed in distilled water for the second period, and again placed in nutrient solution for two or more days*

No.	Days in nutrient solution in third period.	Dry weight.	N	K_2O	P_2O_5
		Gm.	*Gm.*	*Gm.*	*Gm.*
1	Control 0	3.92	0.1596	0.1500	0.0960
2	1	5.88	.2172	.1998	.1320
3	2	5.68	.2320	.2506	.1400
4	3	5.90	.2605	.2670	.1380

By subtracting the nitrogen in the control, 0.1596 gm., from the nitrogen in the 1-day plant (No. 2), 0.2172 gm., we get 0.0576 gm. nitrogen absorbed in one day. By subtracting the control from the 3-day plants, we get 0.1009 gm. instead of three times 0.0576 gm., or 0.1728 gm., which we might expect if time alone governed absorption. In the K_2O column one determination, the absorption in 2 days, seems to be a little out of line and high. From these experiments one would judge that a demand for any of the plant foods can be developed in the plant, that this demand is cumulative, and that it is possible to measure this demand by analytical means.

DEMAND OF WHEAT PLANTS GROWN IN NUTRIENT SOLUTION FULL TIME AND ALTERNATE DAYS

As nitrates, and possibly other plant foods, are likely to vary in the soil solution from day to day, it was thought of interest to measure the rate of absorption when nutrients were given at varying intervals—that is, to measure the rate of absorption when cultures were placed in nutrient solution one day and in distilled water the next, and in nutrient solution on the third day and so on, feasting for one day and fasting the next. These seedlings were grown both with an abundance of plant food (100 parts per million each N, K_2O and P_2O_5) and also with a limited amount (10 parts per million). Analyses of the 12-day-old plants are given in Table VII.

TABLE VII.—*Analyses of 100 wheat plants grown continuously in nutrient solutions for 12 days, compared with similar series placed in distilled water every other day*

No.	Treatment.	Dry weight.	N	K_2O	P_2O_5
1	Distilled water control	4.44	0.0924	0.0597	0.0720
2	Nutrient solution 100 p. p. m. full time.	4.68	.1988	.2561	.1080
3	Nutrient solution 100 p. p. m. one day, distilled water one day.	5.36	.2100	.2856	.1280
4	Nutrient solution 10 p. p. m. full time.	5.76	.1932	.2273	.1200
5	Nutrient solution 10 p. p. m. one day, distilled water one day.	5.92	.1736	.1940	.1180

This was repeated several times with similar results. The total quantity of plant food absorbed, when plenty of plant food was present, was greater in the plants that had grown in the nutrient solution only half time than in the plants that had grown all the time in the same nutrient. When a limited quantity of plant food was present, the quantity

absorbed was reduced by removing the plants from the nutrient solution for half the time. But when calculated upon the rate of absorption per day, both the plants in the strong solution and those in the weak had a higher rate when grown only half time in the nutrient solution.

It is somewhat surprising to note that not only the rate of absorption but the general appearance of the plants—color, size, root development, and vigor of growth—is usually better when the plants are grown in a good nutrient solution for one period and in distilled water the next. This is not always the case but seems to be characteristic of plants that are grown during warm weather, when growth and absorption are rapid, and is not so likely to be true of plants grown in cold weather, when growth and absorption are relatively slow. Better looking plants are often obtained when they are kept in the nutrient solution at night and in distilled water during the day.

The experiments here described seem to demonstrate that a demand for plant food may exist within the plant, that this demand may be modified in different ways, and that the demand may be determined by analytical methods. The author has demonstrated the fact that it is possible to go out in the field, to take up a plant and put it in a full nutrient solution, and to determine what plant food it is hungry for by the way it feeds upon the nutrient solution.

TRANSFER OF PLANT FOODS WITHIN THE PLANT

The plant seems to feed upon the ions and not upon the molecules, and no plant seems to require a base and an acid in exactly the proper proportion to form a salt. It has been shown by Breazeale and Briggs [2] that a plant is even particular as to the kind of ion that it absorbs. Plants that had a high demand for potassium when placed in a solution of orthoclase were unable to feed upon the dissolved potassium. The solution was dilute, it is true, but not so dilute as to prevent the absorption of potassium. It is probable that the potassium existed in the solution as a double ion in combination with aluminum. The plant did not need and could not utilize the aluminum and therefore would not take up either the potassium or the aluminum.

That the transpiration rate has little to do with absorption can easily be shown by placing a bell jar over a pan of cultures, cutting down the transpiration, and measuring the rate of absorption in comparison with controls. Plants will feed just as rapidly when transpiration has thus been reduced to a minimum as they will when transpiring a maximum amount of water.

One can scarcely conceive of a plant feeding upon ions or exercising selective absorption in such a decided way if the transpiration stream or the osmotic concentration, or any other phenomenon except the specific demand of the plant, dominates the process of absorption.

In the same way, practical field results indicate that all plants do not possess the same ability to feed when placed in competition with each other. We find that if an oat and a mustard plant be grown in the same pot with a very limited supply of nitrogen, the mustard probably will get nearly all of that plant food and the oat very little. Plants vary widely in their ability to cope with each other when placed in keen competition.

[2] BREAZEALE, J. F., and BRIGGS, LYMAN J. CONCENTRATIONS OF POTASSIUM IN ORTHOCLASE SOLUTIONS NOT A MEASURE OF ITS AVAILABILITY TO WHEAT SEEDLINGS. *In* Jour. Agr. Research, v. 20, p. 615-621. 1921.

An experiment with corn and kafir seedlings seems to throw some light upon this subject. Six treatments in duplicate were run, 12 pans in all, with corn and kafir seedlings growing upon the same disks and in the same nutrient solutions. The disks were about 12 inches in diameter and were like those used in the wheat culture work except that the perforations were larger. The corn seedlings were planted upon one half of the disk while the kafir grew upon the other. There were about 50 corn to 300 kafir plants. These seedlings were placed in the following solutions:

No. 1. Control, distilled water.
No. 2. 2 parts per million each N, K, and P.
No. 3. 5 parts per million each N, K, and P.
No. 4. 25 parts per million each N, K, and P.
No. 5. 50 parts per million each N, K, and P.
No. 6. 100 parts per million each N, K, and P.

They were allowed to grow for 17 days. In each pan, 2,500 cc. of solution were used and the experiments were run in duplicate. For convenience the average of the duplicates is given in Table VIII.

TABLE VIII.—*Nitrogen absorbed by corn and kafir seedlings competing in nutrient solutions of various strengths*

No.	Strength of solution.	Total N added.	Nitrogen absorbed.	
			By corn.	By kafir.
		Gm.	*Gm.*	*Gm.*
1	Water	0	0	0
2	2 p. p. m.	0.0450	0.0466	[1] 0.0073
3	5 p. p. m.	.1125	.1094	[1] .0091
4	25 p. p. m.	.5625	.2475	[2] .0180
5	50 p. p. m.	1.1250	.3344	[2] .0504
6	100 p. p. m.	2.2500	.3821	([3])

[1] Loss. [2] Gain. [3] Poor plants.

From this one experiment the indications are that when corn and kafir are placed in keen competition as in solutions containing 2 and 5 parts per million the corn may get all the nitrogen and the kafir little or none. An actual loss of nitrogen is noticed in the kafir in the two lowest concentrations, which might be explained by the exudation of this element into the solution or by the probable error of the experiment. The solutions were well mixed and not stirred while the plants drew out the nitrogen. In places the kafir roots were 6 inches away from the corn and in contact with the nitrates in the solution but, the kafir being a sluggish feeder while the corn was a vigorous feeder, the nitrates seem to have gone to the corn and not to the kafir. The absorption of the small quantity of nitrates was so rapid that it does not seem reasonable to assume that diffusion carried it all to the corn side of the pan. The difference is so marked that one must admit that in this case the kafir had no chance in competition with corn and that the nitrogen must have moved as far as 6 inches in a very short time. If, in a soil at optimum moisture content, the plant is dependent upon the soil grains that touch its roots, this competition is rather difficult to conceive of. Practically speaking, the absorbing surface of the roots of different plants would hardly touch the same soil grains often enough to be of serious consideration.

MOVEMENT OF PLANT FOODS IN THE SOIL

Ions are mobile and bear plus or minus charges of electricity. It has long been the opinion of the writer that the demand for food originates in the tissues of the plant and is carried to the absorbing surface of the root by means of an unsaturated carbon compound bearing a plus or minus charge. In the case of potassium, for example, the plant protoplasm in a leaf cell may remove an atom of K from a colloidal compound and use it in building up a permanent compound, which is to be one of the final constituents of its tissues. The removal of the atom K, bearing a plus charge, from its colloidal compound leaves that compound or molecule out of equilibrium and with a minus charge. This charge is transmitted, by replacement and not by bodily movement, down through the cells to the root tips and there appears as a minus charge. If potassium chlorid appears in the nutrient solution ionized as a plus K and a minus Cl, the plus ion will be attracted to the negative charge, and a chemical combination will take place, with the formation of a molecule in equilibrium, with respect to plus and minus electrictity. The potassium could, in this way, be transported from the absorbing surface of the root to the extreme tips of the plant without a bodily movement through the sap. In the case of nitrates the opposite conditions might prevail. A demand for NO_3 might originate in the tissues and be carried to the root as an unsaturated compound bearing a plus charge. This would be neutralized, for example, by the NO_3 ion of $NaNO_3$.

It is possible that the absorbing surface of the root or its walls are actually impermeable to the salts needed in nutrition, and it is possible to assume that food material may be transported from the roots to the rest of the plant without materially affecting the osmotic pressure of the sap. If the food materials are flowing freely in the sap and were it necessary for the plant to remove these materials in the localities where they were needed, it seems probable that there might be times when a high demand and a low supply, or the reverse, might cause considerable fluctuation in the concentration of the sap. As the writer understands it, the sap of plants of like varieties grown in the same localities is fairly constant. The tendency of the plant seems to be to keep its solutions in equilibrium, and the writer can conceive of a plant acting much like the battery of an automobile—the needle of the indicator may register a charge at one instant and a discharge at the next. The plant probably vibrates around the equilibrium point as closely as possible, taking up a plus charge at one time and a minus charge at another. The ordinary plant uses more of the mineral bases than it does of the mineral acids, and the writer is fairly well convinced that in order to maintain equilibrium the plant can absorb otherwise useless acids or bases, use them as ions when necessary, and eliminate them in various ways. With certain plants, if the system is basic, they seem to absorb CO_3 as an acid radical for the purpose of maintaining equilibrium; and in case the system becomes too acid they seem to possess the power of exuding the carbonates as CO_2. The absorption of calcium by certain plants and its elimination as an oxalate might be explained in this way. The absorption of silica in large quantities may probably be traced to the presence of an excess of basic radicals in those plants. The plants that have this characteristic have usually had waterlogged marsh lands for their habitat, and in the ages of their adaptation have had a large quantity of soluble silica and a small quantity of carbon dioxid at their

disposal and as a matter of necessity have adapted themselves to the former.

The plant seems to feed upon ions, and these ions are certainly mobile. If an ionic movement takes place between the root tips and the rest of the plant, it seems equally reasonable to assume the possibility that a similar movement might exist outside the plant and that the plant might draw its food from relatively long distances.

The potassium concentration of young plants, sometimes having small root systems, often runs very high. The soil solution is relatively low in this element, and water movement and diffusion are neglible factors. The absorbing surface of the root is small, and, assuming that the root is obliged to come in contact with a soil grain before it can draw upon the potassium, it is rather difficult to account for the high potassium content of the plant. In the same way the writer has analyzed many samples of Australian saltbush that ran 8 per cent or more of sodium chlorid upon the basis of their dry weight. These plants had grown in a soil that was very low in sodium chlorid and in a semiarid climate where the soil moisture was very low for the greater part of the year. It seems hardly possible for a plant with growing habits like that of the saltbush to be able to absorb such quantities of salt as it does, if obliged to feed in the manner usually attributed to plants. It also seems hardly possible to conceive of this plant being forced to absorb sodium chlorid against its will, so to speak, because the salt is carried into the system by the transpiration stream or other agencies. A noticeable feature of this sodium-chlorid absorption is that the sodium (Na) is absorbed in much larger quantities than are necessary for combination with chlorin (Cl) in the formation of sodium chlorid (NaCl). The excess sodium exists in the plant in organic combinations and is broken down into carbonates upon ashing. Evidently there exists in the Australian saltbush a demand for sodium and chlorin, and the demand for sodium is greater than the demand for chlorin. This absorption of the sodium ions in excess of the chlorin, from a soil solution where the source of supply of these ions is sodium chlorid, which is in equilibrium, seems to eliminate the idea of "forceful feeding," as applied to this plant. The high salt content of the plant, with such a low transpiration, would also indicate a wider field of absorption than is usually attributed to it.

If a soil solution is in equilibrium and the salts are ionized and the ions are mobile, if an atom of the K, for example, bearing a plus charge is removed from solution by the root, the writer sees no reason why the position of this ion can not be filled by replacement and the charge carried along through the soil, as it is in a battery, to where the source of supply of potassium exists. If this be true, the plant will not be dependent upon the soil grains that touch the root tips, but it may actually feed at a distance, the distance probably following some well-known physical law. In practical agriculture we, involuntarily, think of the plant as having all the moist soil surface at its disposal. We also think that the water movement in the soil is neglible as far as nutrition is concerned, that the plant has to grow for its water, and that diffusion is also a neglible quantity. With plants that have a limited root system and with the absorbing zone of the root but a small part of the root itself, if we do not attribute to the plant the ability to feed at a distance, we will have to admit that only a small part of the soil is at the disposal of the plant.

CONCLUSIONS

(1) A demand for plant food may be developed in the tissues of plants, and this demand may be measured by analytical methods.

(2) The demand of the plant seems to be for particular foods, and the effect of an application of plant food as a fertilizer seems to be largely a direct action upon the plant itself and not an indirect action upon some constituent of the soil.

(3) Plants probably feed upon ions, and these ions probably penetrate the root membrane and move through the colloids to the tissues as an electrical charge; therefore the feeding of plants may be looked upon as an electrical phenomenon.

(4) Ions are mobile and may move through the soil solution freely as such; and, this being the case, the plant may not be dependent upon the soil grains that touch its roots for nutrient material but may feed at a distance from the source of supply.

INFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE UPON THE FUSARIUM DISEASE IN CABBAGE SEEDLINGS [1]

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INTRODUCTION

The pioneer investigations concerning the influence of soil temperature upon cabbage yellows caused by *Fusarium conglutinans* Wollenw. were conducted in Wisconsin by Gilman (*8*).[3] His experiments were conducted in greenhouse rooms with rather wide fluctuations of air temperature, where the soil temperature could not be held constant for any great length of time. Consequently, Gilman did not determine the complete range of soil temperature for the occurrence of the disease, nor did he inquire into the possible influence of soil moisture.

Since the publication of Gilman's paper, plant pathologists have continued to make observations on the influences of air and soil temperatures upon the growth of the plants and upon the occurrence of yellows in the Wisconsin cabbage fields. During the midsummer months when the soil is dry and hot, cabbage plants begin to languish and assume a pale, lifeless color. Growth is markedly checked, especially when these conditions obtain for two or three consecutive weeks. It is during this trying period that yellows develops in its most destructive form on the "cabbage sick" [4] soils. During very dry hot seasons even the resistant strains of cabbage, such as the Wisconsin Hollander, may show a considerable percentage of incipient disease, but upon the return of more favorable weather conditions (rain and lower temperature) they usually overcome the attack and produce marketable heads. Such field observations soon convince one that the occurrence and severity of yellows are closely correlated with the influences of soil temperature and soil moisture, and the presumption would seem to be that these influences relate both to host and to parasite.

The writer undertook to learn more exactly the importance of these factors as they affect young seedlings, beginning the work in the summer of 1917. The purposes outlined were: (1) To determine the range of soil temperature for the occurrence of yellows in cabbage seedlings; (2) to study the influence of such soil temperatures upon the normal growth of cabbage seedlings in noninfested soil; (3) to study the influence of high soil temperature upon the relative susceptibility shown by the resistant strain, that is, upon the possible "breaking down" of resistance; (4) to determine in a like manner the influence of soil moisture both upon the growth of cabbage plants and upon the occurrence of yellows in them.

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[1] Accepted for publication Jan. 5, 1922.

[2] The writer wishes to make grateful acknowledgment to Prof. L. R. Jones, of the University of Wisconsin, for supervision and helpful suggestions during the progress of this work.

[3] Reference is made by number (italic) to "Literature cited," p. 86-86.

[4] The term "sick" or "cabbage sick" soil as used in this paper indicates soil infested with *Fusarium conglutinans*.

Cabbage seedlings have been found to lend themselves fairly well, distinctly better than the larger plants, to these studies. The reasons for this are that (1) the symptoms of the disease become evident very soon after the seedlings emerge from the soil and diagnosis is easy and sure for the experienced observer; (2) the experiments can, therefore, be carried to definite conclusions within a relatively short time; (3) for such a brief period the seedlings develop well in fairly crowded plantation so that adequately large numbers can be used in pot culture; (4) the seedlings behave well under a reasonably wide experimental range of variation in soil temperature and soil moisture.

INFLUENCE OF TEMPERATURE UPON THE GROWTH OF FUSARIUM CONGLUTINANS IN CULTURE

A partial knowledge of the influence of temperature upon the growth of *Fusarium conglutinans* has already been obtained. Gilman (*8*) in his early work found that the conidia did not germinate within 72 hours when exposed at 8° to 10° C. in Van Tieghem cells, while only 3 hours were required for germination at 33° and 8 hours at 21°. His study of the growth of the mycelium was limited to a narrower range of temperature. The mycelium grew slowly at 8° to 10° and most vigorously at 25°, the highest temperature used in his series. An intermediate growth rate was obtained at 18° to 22°. It is seen that data on the upper limits of temperature were still lacking; therefore, it seemed advisable to determine these limits before taking up a study of the relation of soil temperature to the occurrence of yellows.

A fragment of mycelium from a young culture of *Fusarium conglutinans* was placed in the center of plates (10 by 100 mm.) of 2 per cent potato-dextrose agar, titrating + 10 Fuller's scale. The plates were then placed in incubators at a series of temperatures ranging from 7° to 37° C., those at the higher temperatures being inclosed in moist chambers to guard against inhibiting desiccation. Two plates were carried at each temperature, and measurements were made daily for seven days. The results at the end of four days and those at the end of seven days are given in Table I and are also shown graphically (fig. 1) for comparison with the percentage of yellows.

TABLE I.—*Growth of Fusarium conglutinans at different temperatures on potato agar*

Temperature.	Diameter of colony at various ages.	
	4 days.	7 days.
°C.	*Cm.*	*Cm.*
7 to 8	No growth.	Slight growth.
11 to 12	0.5	1.6
17 to 18	1.3	2.9
21 to 22	1.6	3.5
24 to 25	3.2	6.0
27	3.3	6.0
30	1.2	2.0
35	.3	.6
37	No growth.	No growth.

It is readily seen that if diameter of colony is used as a criterion, the optimum temperature for growth on potato agar plates for brief periods of time is found to be between 24° and 27° C., with a rather sudden dropping off toward the extremes. There was no growth at 37°, but the fungus was not killed at this temperature, as was shown by transferring the plates to an incubator held at 24°. After exposure for 10 days at 24° the colonies on the plates which had previously been exposed at 8° and 37°, respectively, showed vigorous growth.

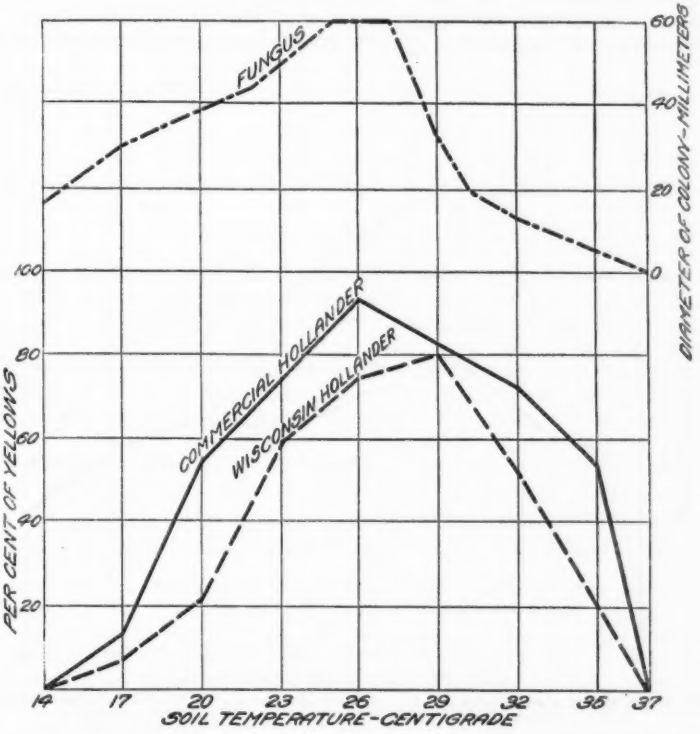

FIG. 1.—Comparison of rate of growth of *Fusarium conglutinans* on agar plates with development of yellows in cabbage seedlings given in Tables I and V; plants grown 20 days from seed in naturally infested soil.

In addition to these differences in vegetative growth, the fungus exhibited a difference in sporulation at the different temperatures. At 14° C. the colonies were raised, with a leathery stroma, and produced abundant conidia. This character became less manifest up to 24°, where there was very little aerial growth and there were practically no conidia. Above 29° the colonies were again raised and produced abundant chlamydospores but no conidia. Table I shows that the growth of the fungus dropped off more suddenly above 27° than below, yet it will be seen (fig. 1) that the percentage of yellows was higher at 35° than at 17°. It is evident, therefore, that rate of growth of the fungus in culture is not directly proportional to percentage of disease at all temperatures.

Although the vegetative growth of the parasite is retarded at the higher temperatures, the data do not indicate that the pathogenicity is reduced in like proportion.

INFLUENCE OF SOIL TEMPERATURE UPON THE GROWTH OF CABBAGE SEEDLINGS

Inasmuch as it was presumed at the outset that soil temperature affects both host and parasite, it was considered of much importance to study separately its effect upon the cabbage plants in noninfested soil as well as upon the fungus in culture. The results of the studies of the influence of temperature upon the fungus have already been presented.

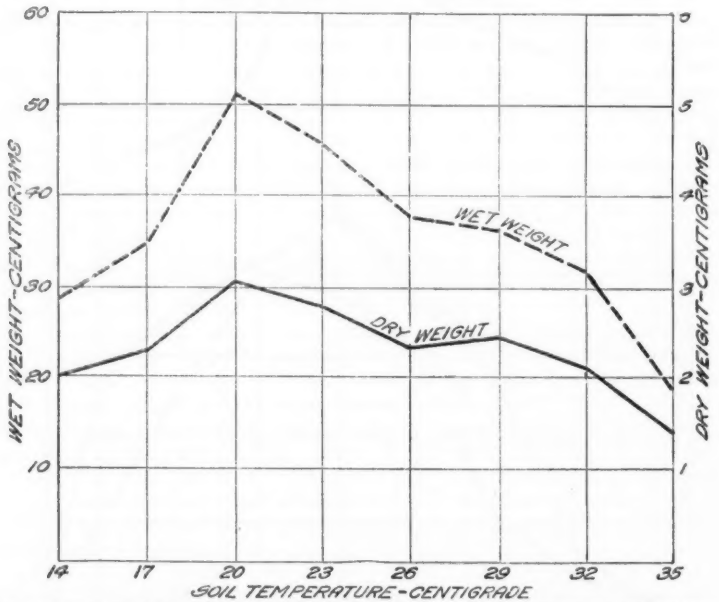

FIG. 2.—Comparison of wet weights and dry weights of shoots of healthy Wisconsin Hollander cabbage seedlings given in Table II, grown 20 days from seed during February and March.

In the experiments conducted for the purpose of determining the influence of soil temperature upon the seedlings, the general appearance of the plants and the dry weight of the shoots and roots were used as bases for comparison. For preliminary data use was made of the Wisconsin Hollander plants which had been grown 20 days in sterilized soil as controls on the relation of soil temperature to the occurrence of yellows. The methods of planting and controlling the soil temperature will be explained in connection with the experiments for studying the influence of soil temperature upon the occurrences of yellows. At the end of 20 days six plants were taken for each temperature and both wet and dry weights were determined. The tops were cut off at the surface of the soil and dried in a constant-temperature oven for 18 to 24 hours at 95° C. The average results of two such series are given in Table II and shown graphically in figure 2. These first trials were conducted during February and

March when the light was weak and the day short. Other experiments were carried out in May and June when the light was stronger and the plants were grown for a longer period of time.

TABLE II.—*Weights of Wisconsin Hollander seedlings grown 20 days from seed at different soil temperatures*

Soil temperature.	Wet weight per plant.	Dry weight per plant.	Ratio of wet weight to dry weight.
°C.	Gm.	Gm.	
14.5	0.29	0.020	14.5
17.0	.34	.023	14.7
20.0	.51	.031	16.4
23.0	.45	.029	15.5
26.0	.37	.023	16.0
29.0	.36	.024	15.0
32.0	.32	.022	14.5
35.0	.18	.014	12.8
38.0	.06	.006	10

It will be seen from Table II that 20° C. proved to be the optimum soil temperature for the growth of these cabbage plants for the first 20 days. At soil temperatures above 20° there was a gradual decrease in weight up to 35°, where there was a sudden drop; at 38° the plants soon died. Accompanying this difference in weight was a marked difference in the appearance of the plants grown at low and at high temperatures. At 23° and below they were stocky and had a dark green color. There was also a slight difference in the height and size of the plants at the different temperatures. From 26° to 32°, inclusive, the plants became more strict; that is, the petioles were proportionately longer and approached a vertical position. The color also graded into a lighter shade of green. At 35° the plants were decidedly stunted, and they assumed a distinctly pale green color.

Moreover, if we consider the ratio of wet weight to dry weight, a marked difference will be noticed at the different soil temperatures. At the intermediate temperatures, where the greatest wet and dry weights developed, the ratio of the wet weight to dry weight was greatest. This simply means that the plants were most succulent at these temperatures, and it seems possible that for this reason they may have offered a more favorable medium for the invasion of the parasite than was offered by the plants at the extreme temperatures. This suggestion is favored to some extent by the occurrence of a higher precentage of yellows in plants growing in naturally infested soil at these temperatures. On the other hand, as will be seen later, the highest percentage of yellows occurs in plants grown in artificially inoculated soil above 26° C., where the moisture content of the plant is lower and the plants must be in an abnormal condition. These results indicate that some factor other than succulence of the host plant must be concerned in determining the degree of infection.

In order to determine whether similar temperature relations obtained for longer periods, the seedlings used as controls in the third experiment in studying the disease were allowed to grow at the different soil temperatures for 46 days before the weight determinations were made, as compared with 20 days in the former series. The roots were then washed

out of the soil and dry weights were made separately for roots and shoots. The results, consisting of the average per plant for the nine plants from each temperature, are shown in Table III. This experiment was conducted in May and June, 1919, when the air temperature ranged from 18° to 20° C., with a rise of 10° to 12° during the middle of the day.

TABLE III.—*Dry weights of cabbage plants grown 46 days from seed at different soil temperatures*

Soil temperature.	Dry weight per plant.			
	Wisconsin Hollander.		Commercial Hollander.	
	Shoot.	Root.	Shoot.	Root.
°C.	Gm.	Gm.	Gm.	Gm.
14	0.245	0.029	0.185	0.027
17	.316	.033	.306	.048
20	.281	.042	.262	.041
23	.272	.044	.223	.037
26	.254	.041	.192	.025
29	.302	.052	.252	.035
32	.236	.030	.260	.036
35	.207	.025	.204	.017

It may be seen from Table III that the weights are not consistent at all temperatures with those of plants 20 days old, although the temperature for optimum growth remains the same. The weight at 26° C., is less than that at 23° and 29°, thus giving a distinct bimodal curve when the weights are plotted against temperature. This condition exists alike with roots and shoots of both strains.

Inasmuch as the moisture content of the soil was not kept constant by weight, it was thought that a difference in moisture, provided there were such, might have been responsible for the irregular growth of the plants. Therefore, another experiment was conducted, during the winter of 1919, in which the whole soil mass was kept up to a uniform moisture content by weight. The soil was made up of three parts of fairly rich, virgin loam and one part of clean sand. The water-holding capacity of this soil was 39.2 per cent (in 1 cm. tube), or 28 per cent calculated on a wet-weight basis, and the moisture was kept at about 15 per cent by weighing the receptacles at the higher temperatures every day and less frequently at the lower temperatures and restoring the lost moisture. In place of the cinders used in the other experiments, a 3-inch pot was inverted in the bottom of the receptacle, a glass tube inserted in a hole in the bottom of the pot, and the soil filled in around this and tamped fairly firmly. All of the water was supplied through the tube. The seed was planted as previously described. After the plants were about 5 days old, the stand was thinned to three in each receptacle, and the soil surface was covered with mineral wool. Both the Wisconsin Hollander and the Commercial Hollander were used, the receptacles being handled in duplicate at each temperature.

The experiment was begun October 22 and concluded December 15, 1919, a total of 53 days. The air temperature during this period ranged from 14° to 18° C., with a daily rise of about 5° in the middle of the day.

The roots were washed out of the soil and dry weights made of both shoots and roots in the usual way. The results in Table IV show the average of six plants from each temperature. These results are shown graphically in figures 3 and 4.

TABLE IV.—*Dry weights of cabbage plants grown 53 days from seed at different soil temperatures*

Soil temperature.	Wisconsin Hollander.		Commercial Hollander.	
	Shoot.	Root.	Shoot.	Root.
°C.	Gm.	Gm.	Gm.	Gm.
14	0.230	0.034	0.267	0.039
17	.305	.036	.313	.046
20	.302	.036	.313	.040
23	.239	019	.236	.020
26	.258	.021	.255	.019
29	.200	.020	.309	.024
32	.185	.017	.233	.012
35[a]	.021	.006	.053	.005

[a] The temperature rose to 38° C. during two successive nights soon after the plants appeared above ground; this may account for the extremely low weights occurring at this temperature.

This table shows that 17° to 20° C. was also the optimum soil temperature for the growth of cabbage seedlings for this longer period. The dry weights obtained at some temperatures were even lower than the weights

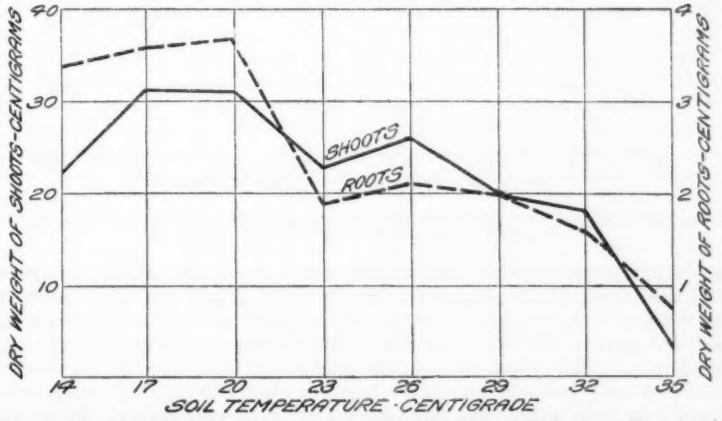

FIG. 3.—Comparison of dry weights of shoots and roots of healthy Wisconsin Hollander seedlings given in Table IV, grown 53 days from seed during November and December.

obtained for plants only 46 days old, but the difference in the composition of the soil was probably sufficient to account for the difference in weight. The bimodal condition was evident in this experiment also, but in this case the lowest weight occurred at 23° instead of 26°, as in the previous experiment. The bimodal condition in weight and the difference in color were probably due to the effect of temperature upon the physiological balance of the plants, but since no analyses have been made of plants

grown at the different temperatures, it is impossible to offer any definite explanation for these differences. However, by comparing figure 1 with figures 3 and 4, it will be seen that the higher percentages of yellows occurred at and slightly above the temperature at which the drop in dry weight of the plant occurred, which was also the temperature at which the fungus grew most rapidly in culture. It is probable that both reduced vigor of the plant and optimum temperature for growth of the fungus played a part in producing a higher percentage of yellows at these temperatures.

There is a marked difference in the external characters of the root systems of the plants grown at different temperatures. Roots grown at 14°, 17°, and 20° C. are coarse, light in color, have a thick cortex, and spread uniformly throughout the soil mass. Beginning at 23° the roots

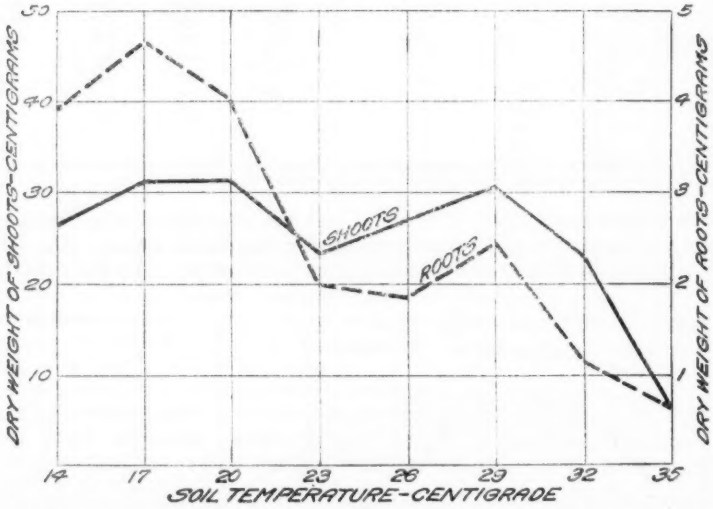

FIG. 4.—Comparison of dry weights of shoots and roots of healthy Commercial Hollander seedlings given in Table IV, grown 53 days from seed during November and December.

are finer, darker in color, and shorter, until at 35° the number of roots and the extent of the system are greatly reduced and the color becomes dark brown. A comparison of the shoots and roots of plants grown at 20°, 26°, and 32° is shown in Plate 1. All of these variations suggest that fundamental differences occur in the anatomical structures of the tissues involved and possibly in their chemical composition, but analytical studies on these differences have not as yet been undertaken. It seems evident, however, that such variations in external appearance of parts of the host at different temperatures may be regarded as indices of the more important internal effects of temperature. They are probably accompanied by a difference in the rate of manufacture of carbohydrates and proteins and in the disposition of these products. In turn, these differences in assimilation and physiological balance could affect the cell walls and protoplasm in a manner which would make the plant more susceptible to the attacks of the fungus.

INFLUENCE OF SOIL TEMPERATURE UPON THE OCCURRENCE OF THE FUSARIUM DISEASE IN CABBAGE SEEDLINGS

GREENHOUSE EXPERIMENTS

METHODS AND APPARATUS.—The apparatus employed for controlling the soil temperature in these experiments was what is termed the Wisconsin soil-temperature tank. Since it differs only in detail from that used by Johnson and Hartman (*9*) and described by Jones (*10*), further description is considered unnecessary in this connection. A series of nine separate tanks or compartments was employed, with extreme temperatures of 14° and 38° C. and a difference of 3° between successive tanks, thus giving the graduated series 14°, 17°, 20°, 23°, 26°, 29°, 32°, 35°, and 38°. The temperatures below 23° were regulated by running in a small amount of cold water two or three times a day as the case required, while the temperatures from 23° to 38° inclusive were maintained through the use of carbon electric lamps under thermostatic control. During the earlier experiments some of the temperatures were regulated through the use of steam, but this method was abandoned when the electric lamps were installed. With personal attention two or three times every 24 hours, the temperatures were kept fairly constant, rarely varying more than 1° from that desired.

The air temperature in the tank room was kept constant within a range of a few degrees. During the course of the first experiments it ranged from 18° to 22° C. with a maximum rise of 5° to 10° for one or two hours on bright days. During the spring of 1919 the air temperature range was from 14° to 18° with a similar rise at midday on bright days.

The surface of the soil in the receptacles was covered with a half-inch layer of mineral wool to insulate it from the air temperature and to reduce evaporation. Even with this protection, the surface inch of soil at the higher temperatures usually registered from 1° to 1½° lower than the water in the tanks. However, the deeper layers of soil registered the same temperature as the water, and the plant roots were found to be distributed largely in the layers below the first inch. Therefore the temperatures recorded for the different series are those at which the water was maintained in the tanks in which the receptacles were suspended.

RECEPTACLES.—The receptacles or culture pots were made of galvanized sheet iron, cylindrical, 6 inches in diameter and 10 inches deep. They were made ready for use by placing a layer of fine coal cinders about 2 inches deep on the bottom with soil on top of this up to within 1½ inches of the top. All of the water was supplied through a glass tube 12 mm. in diameter which was placed in the center of the receptacle of soil with the lower end inserted in the cinders. After the receptacles were filled with soil, they were so placed in the tanks that the surface of the soil was level with the surface of the water in the tank. They were then allowed to remain in the tanks one or two days before the seed was planted.

SOIL.—The naturally infested soil used in all the experiments was obtained from a uniformly "cabbage sick" field in Kenosha County, Wis. It was a dark clay loam containing some gravel. Before being used the soil was screened and uniformly mixed with coarse sand in the ratio of five parts of soil to one of sand. After the sand was added, the portion to be used as control was autoclaved at 5 pounds pressure for two hours and then allowed to stand for one week before it was used.

HISTORY OF SEED AND METHODS OF PLANTING.—The seed used in all the experiments was from the same lots of the Late Wisconsin Hollander and Commercial Hollander strains. The Late Wisconsin Hollander was the strain of Hollander which has proved to be highly resistant to the Fusarium disease under field conditions, as recently described by Jones, Walker, and Tisdale (*12*). This seed was grown in 1917 by S. B. Walker at Racine, Wis. The seed of the commercial strain was obtained from the L. L. Olds Seed Co., Madison, Wis. No information could be had as to where and when it was grown, but it was considered a good representative of the Commercial Hollander type. It had been proved to be about 99 per cent susceptible when grown in "sick" soil.

In the first and third tests, three receptacles in each tank were planted with Wisconsin Hollander and three with the Commercial Hollander. Two of the three receptacles in each set contained "sick" soil and one contained sterilized soil. In the second experiment only two receptacles were used for a strain in each tank, one containing "sick" and the other sterilized soil. When the plants were well above ground, the stand was thinned to 10 plants in each receptacle, except in certain cases, and the surface of the soil was covered with a half-inch layer of mineral wool.

THE EXPERIMENTS.—Four separate tests were conducted for measuring the influence of soil temperature upon the occurrence of yellows in cabbage seedlings, three with naturally infested soil and one with artificially inoculated soil. Three of the tests were conducted during the winter of 1917 and the spring of 1918, and one in the spring of 1919. In all three experiments with naturally infested soil the behavior of the host plant was similar, and the percentage of disease checked fairly closely. Of course, the intensity of light was greater in the spring than during the winter, but it was reduced in the spring by nailing cheesecloth on the inside of the roof of the greenhouse above the tanks. The final data were recorded 20 days after the seed was planted; they show the percentage of yellows and the percentage of plants dead by this time. This length of period was chosen because in the first experiment all of the plants of the commercial strain at 32° C. had developed yellows within 20 days.

METHODS OF EXPRESSING DATA.—The data in the greenhouse experiments were taken daily after the disease began to develop. In the first four experiments the task of taking data consisted merely in recording the number of diseased plants and the number which died as a result of the disease. The percentage of yellows includes the plants which showed the disease in incipient stages as well as those which died from the disease. Except in doubtful cases diagnoses of yellowed plants were made from the external symptoms in the leaves; the doubtful cases were cultured on agar plates.

EXPERIMENTAL DATA.—The data given in Table V and figures 1 and 5 show that *Fusarium conglutinans* is capable of producing yellows in both the susceptible Commercial Hollander and the resistant Wisconsin Hollander seedlings over a wide range of temperature, the minimum being 17° and the maximum about 35° C. At the lower temperatures, however, the disease developed more slowly and less destructively than at higher temperatures (fig. 6). The Wisconsin Hollander strain was even less severely attacked at the lower temperatures than the commercial strain. Plate 2, A–C, shows a contrast of the two strains growing in "sick" soil at 17°. At 15° the disease did not develop even in the most susceptible strains. The optimum soil temperature for the occurrence of yellows in the commercial strain in naturally infested soil

was about 26° and in the Wisconsin Hollander about 29°. These temperatures practically coincide with that for growth of the causal organism on potato agar, but are several degrees higher than the optimum for growth of the seedlings. These results differ from those reported by Tisdale (*13*) for flaxwilt and those reported by Clayton (*5*) for tomato wilt only in that the optimum temperature for growth of the cabbage plant is lower than that for development of the disease and growth of the parasite. The same temperature relations do not obtain for other types of diseases attacking the underground parts of plants. Balls (*1*, *2*) reported that the soreshin disease of cotton developed most destructively at 17° to 23° C., while the optimum for the growth of host and parasite was about 28°. Johnson and Hartman (*9*) reported quite similar relations for the rootrot of tobacco.

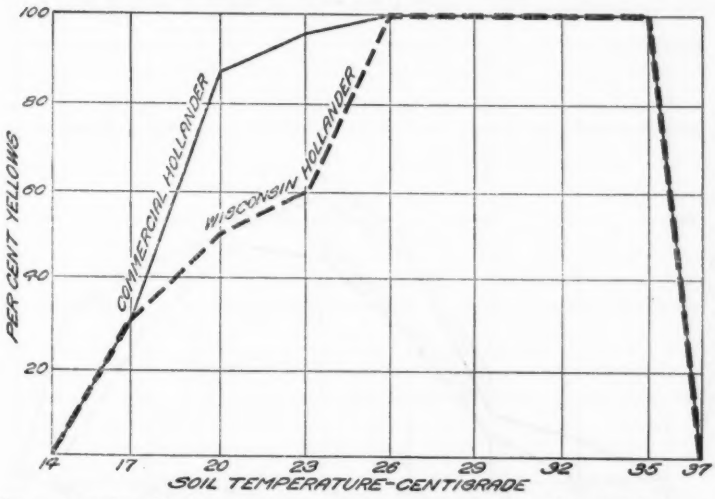

FIG. 5.—Comparison of development of yellows in Commercial Hollander and Wisconsin Hollander seedlings given in Table V, grown 20 days from seed in artificially inoculated soil.

Although the percentage of yellows in the resistant Wisconsin Hollander was rather high during the early period of growth, it was lower at all temperatures than that of the susceptible commercial strain. The greatest differences in percentage of yellows between the two strains occurred at 20° and at 35° C., where they amounted to about 35 per cent. In artificially inoculated soil at 26° and above the two strains seemed to be equally susceptible (fig. 5). The cause for such a wide difference between the pathogenicity of *Fusarium conglutinans* in naturally and in artificially inoculated soil has not been determined. It is apparent, however, that this difference is in part due to the direct relation of the higher temperatures to the stimulation of the Fusarium. This relation becomes especially evident when the fungus is growing in pure culture free from the complicating relations with the normally associated soil flora.

Other workers have obtained similar results with vascular parasites. Edgerton (*6*, *7*) found that a much higher percentage of Fusarium-wilt of tomato developed in the sterilized, reinoculated soil than in the unsteril-

ized soil, but at the same time the resistant tomato was very resistant in the seedling stage. He did not state the temperature at which the plants were grown, however, and temperature is a potent factor in the development of cabbage yellows. At 23° to 32° C. seedlings of the resistant strain of cabbage show a high percentage of yellows, but the plants which escape the disease usually remain healthy. W. H. Tisdale (*14*) found the resistant strain of flax to be highly resistant even in the seedling stage.

The upper limit for the growth of *Fusarium conglutinans* in pure culture is about 35° C., yet it produced 100 per cent yellows in plants grown at this temperature in artificially inoculated soil. As previously stated, the temperature of the first inch of soil at 35° registers from 1° to 1½° lower than the water in the tank, and it may be that the fungus made its successful attack upon these roots in the first inch of soil.

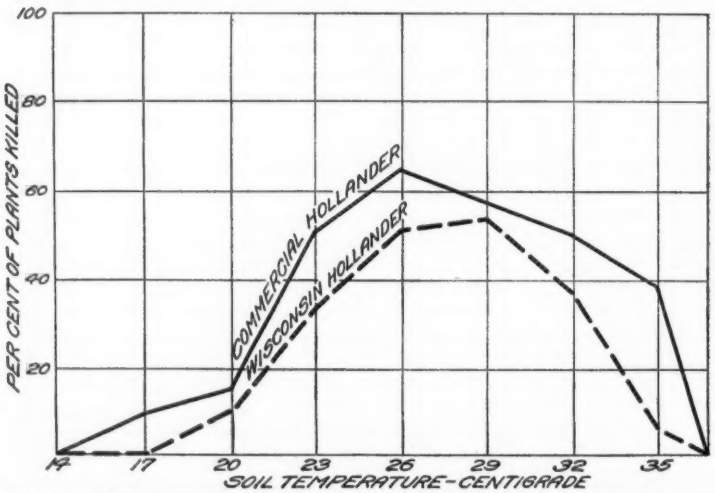

FIG. 6.—Comparison of death rate from yellows in Commercial Hollander and Wisconsin Hollander seedlings given in Table V, grown 20 days from seed in naturally infested soil.

The data presented in Table V and figure 6, especially those for artificially inoculated soil, show that the highest percentage of yellows occurred at temperatures above that most favorable for the seedling. This indicates that resistance is probably broken down at these higher temperatures. However, the chief difficulty in determining to what extent the increase in percentage of yellows at higher temperatures is due to "breaking down" of resistance is the fact that the nature of the resistant character has not been analyzed. Until its true nature has been determined, it will be impracticable to learn by the common method of testing for resistance in plants, that is, by subjecting both host and parasite to the different temperatures, just how much temperature affects the resistant character. However, the results obtained with artificially inoculated soil indicate that either the host becomes less resistant or the fungus more pathogenic at the higher temperatures. The latter condition was not in evidence with susceptible plants, but the decided stunting of the plants would seem to indicate that they may be less resistant.

TABLE V.—*Percentage of cabbage seedlings showing yellows at different soil temperatures after growing 20 days from seed*

Condition of soil.	Strain.	Date planted.	Soil temperature. 14° C.			17° C.			20° C.			23° C.			26° C.			29° C.			32° C.			35° C.			38° C.		
			Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.[a]
Naturally infested	Wisconsin Hollander	Feb. 1	20	0	0	19	11	0	19	22	11	20	80	60	20	80	50	20	90	60	20	70	50	10	20	0	50
		Mar. 2	18	0	0	20	0	0	20	20	10	20	50	20	20	80	20	20	50	20	20	60	20	20	17	0	30
		Mar. 29	10	0	0	10	10	0	10	20	0	10	50	30	10	70	20	10	100	80	10	40	40	10	10	0	40
	Commercial Hollander	Feb. 1	19	0	0	17	14	14	18	67	25	17	71	57	19	78	50	20	80	50	20	100	100	100	17	57	29	15	0	60
		Mar. 2	16	0	0	17	14	0	19	67	11	19	78	67	17	100	100	18	75	50	22	59	40	21	36	18	24	0	0	50
		Mar. 29	10	0	0	10	10	0	20	20	10	10	70	30	10	100	50	10	90	70	10	60	40	10	70	70	10	0	0	40
	Av. Wisconsin Hollander		48	0	0	....	7	0	....	21	7	....	60	37	....	76	30	....	80	53	....	57	37	....	20	7	....	40		
	Av. Commercial Hollander		....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....		
Artificially inoculated	Wisconsin Hollander	Mar. 29	45	0	0	....	13	8	....	54	11	....	73	50	....	93	65	....	82	58	....	73	48	....	54	40	....	50		
	Commercial Hollander	Mar. 29	10	0	0	10	30	0	10	88	10	10	60	40	10	100	100	10	100	100	10	100	100	15	100	100	10	0	0	50
		May 5	18	0	0	13	31	0	16	88	44	24	96	83	18	100	100	18	100	94	16	100	100	13	100	91	11	0	0	50

[a] The death of the plants at this temperature (38°) was evidently due to the effect of high temperature alone. No culture of Fusarium was ever obtained from the plants.

INCUBATION PERIOD OF THE FUSARIUM DISEASE IN CABBAGE SEEDLINGS AT DIFFERENT TEMPERATURES

In all of the experiments reported in this paper a great difference in the incubation period of the disease was observed in the plants grown at the different soil temperatures, and also to some degree between plants in the same receptacle. The incubation period, as spoken of in this connection, was reckoned from the time the seed was planted rather than from the date the plants emerged from the soil, because at temperatures below 23° C. two or three days more were required for the plants to emerge from the soil than at the higher temperatures, although the seed coats ruptured practically as soon at the lower temperatures. Because of this fairly uniform breaking of the seed coats, the seedlings at the different temperatures were exposed to the fungus for about the same length of time.

In every case the disease appeared first at 26° to 32° and last at 17° C., with a gradation between these extremes. The incubation periods at these temperatures were 7 days and 18 days, respectively. At 26° to 32° the disease appeared in some plants within 1 or 2 days after the plants emerged from the soil, while others in the same receptacle appeared perfectly normal and healthy for several days longer. At the lower temperatures the disease was much longer in becoming manifest but at the same time showed a similar variation in length of incubation among individuals. The variation among plants grown in artificially inoculated soil was not as great as that among plants grown in naturally infested soil. A comparison of the results in the two cases is shown in Tables VI and VII. Gilman (*8, p. 43, Table VI*) also showed that, although inoculations were made with parts of the same culture on plants from the same pot and under conditions as nearly identical as possible, the incubation period varied widely. The cause of this variation has not been definitely determined, but the results so far obtained indicate that it is a difference in the genetic composition of the individuals. Under the conditions of these experiments the plants must have been exposed to essentially like opportunities for attack by the fungus.

When the plants were grown in "sick" soil for 20 days at 14° and then transferred to 26° C., the incubation period was only 4 to 6 days, although a smaller percentage of disease developed in the resistant seedlings than when the plants were grown from seed in "sick" soil at 26°. This short incubation period is very probably due to the fact that the root hairs were in close contact with the fungus mycelium at the low temperature, and when subjected to a more favorable temperature the fungus immediately began invasion of the roots through the root hairs. The Wisconsin Hollander plants treated in this manner showed the disease mostly in incipient form, whereas the susceptible commercial strain was as susceptible as in the earlier stages of development.

When the plants were grown for 30 to 36 days in noninfested soil and then transplanted to "sick" soil the incubation period was longer in all cases than when the seed was planted in "sick" soil. Under these conditions the incubation period was shortest at 26° C. and longest at 17°, being 11 and 17 days, respectively. It is probable that this longer period was due to the inability of the fungus to make a successful attack until new root hairs developed on the roots.

A COMPARISON OF SUSCEPTIBILITY IN THE WISCONSIN HOLLANDER AND COMMERCIAL HOLLANDER SEEDLINGS

In all of the experiments conducted under controlled temperature and moisture conditions variations in individual susceptibility among plants have been very noticeable in both susceptible and resistant strains. Data were obtained from experiments previously reported by recording each day the percentage of plants in each can showing the disease at the different temperatures. These data are shown for both naturally and artificially infested soils in Tables VI and VII.

TABLE VI.—*Variations in susceptibility among cabbage seedlings grown in naturally infested soil at different soil temperatures*

Soil temperature.	Total number of plants.	Percentage of diseased plants in each receptacle at the end of different periods after planting.									
		Commercial Hollander.					Wisconsin Hollander.				
		10 days.	12 days.	15 days.	18 days.	20 days.	10 days.	12 days.	15 days.	18 days.	20 days.
°C.											
17	10	0	0	0	10	10	0	0	0	10	10
20	10	0	0	0	10	20	0	0	0	10	20
23	10	0	30	30	40	50	0	20	30	40	50
26	10	10	50	60	70	100	20	80	80	80	80
29	10	30	60	60	70	90	10	20	20	30	50
32	10	20	20	30	50	60	10	20	30	50	60
35	10	10	40	50	70	70	0	0	0	20	20
38	7	0	0	0	0	0	0	0	0	0	0

TABLE VII.—*Variations in susceptibility among cabbage seedlings grown in artificially inoculated soil at different soil temperatures*

Soil temperature.	Percentage of diseased plants in each receptacle at end of different periods after planting.											
	Commercial Hollander.						Wisconsin Hollander.					
	Total number of plants.	10 days.	12 days.	15 days.	18 days.	20 days.	Total number of plants.	10 days.	12 days.	15 days.	18 days.	20 days.
°C.												
17	13	0	0	0	0	31	10	10	10	10	30	30
20	16	0	0	0	69	88	10	0	0	20	30	50
23	24	0	21	44	92	96	10	0	10	30	50	60
26	18	6	22	67	94	100	10	40	80	100	100	100
29	18	6	28	89	94	100	10	80	90	90	90	100
32	16	50	75	100	100	100	10	80	90	100	100	100
35	23	26	52	87	91	100	10	60	100	100	100	100
37	11	0	0	0	0	0	10	0	0	0	0	0

Examination of the Tables V–VII shows considerable variation in susceptibility as between individuals at all temperatures. This variation is manifest not only by length of incubation period but also by extent of infection and rate of development of the disease once infection has occurred. At and above 26° C. the incubation period is materially shortened for a great number of individual plants, especially those of the Wisconsin Hollander strain. In naturally infested soil Wisconsin Hollander plants which escape the disease for the first 20 days usually remain healthy, even though they are growing at high temperature. In artificially inoculated soil all of the plants become diseased within the first 20 days as a rule.

Plants of the Wisconsin Hollander strain which showed symptoms of yellows even when very young have been observed to overcome the attack and resume vigorous growth. Their ability to do this was less pronounced in plants grown in artificially inoculated soil. It will be seen from Table VII that a high percentage of plants were killed in the artificially inoculated soil. The sudden death of some plants, the ability of others to overcome the attack, and the ability of still others to escape the disease entirely, furnish evidence of a variation in degree of resistance of the individual plants during the early stage of development. The same condition has been known for several years to exist among varieties. However, still more significant is the fact that such variations in degree of resistance continue to appear as between individuals of the most resistant and most stabilized of the Wisconsin strains.

Our main interest lies, therefore, in the variations in resistance as between individual plants. The facts cited as bearing on this variation indicate that Fusarium resistance in cabbage is due to hereditary factors, probably multiple, and that many of the plants are entirely lacking in one or more of the factors for resistance or possess them in a heterozygous condition. The Wisconsin Hollander strain was selected to resist the Fusarium disease under certain conditions to which the plants are normally subjected in the field. Plants which resist under these conditions may not possess sufficient factors for resistance to enable them to resist under more severe conditions, as evidenced during very hot summers. Because of the fact that the seed plants are grown under conditions which permit free intercrossing, the tendency is for them to remain in this heterozygous condition even when grown in "sick" soil. When the seed plants are grown on "sick" soil, however, the plants which possess an insufficient number of factors for resistance develop the disease and are discarded. Thus, by selecting seed plants from "sick" soil, it is possible to maintain a resistant strain, under the conditions selected, even though it may not be homozygous for resistance.

RELATION OF AGE OF SEEDLINGS TO EXPRESSION OF THE RESISTANT CHARACTER

It has been shown (Table V) that during the first 20 days of development the Wisconsin Hollander plants are practically as susceptible to yellows at the higher temperatures as the susceptible commercial strain. On the other hand, the Wisconsin Hollander strain shows a high degree of resistance by the time the plants are old enough to be transplanted into the field. Since this condition has manifested itself for several seasons and since the plants are usually grown in soil with a fairly low tem-

perature before they are transplanted, there appear to be only two possible explanations for the low degree of resistance in the young seedlings, namely: (1) the resistant character is not fully expressed in the young seedlings and becomes more manifest as the plants grow older, or (2) the high temperatures inhibit or modify the expression of the resistant character in seedlings. Experiments have furnished some evidence favorable to both hypotheses. Data supporting the second have already been presented and discussed.

The initial experiments for testing the first theory were conducted by transferring the receptacles to the 26° C. tank after the plants had grown 20 days in infested soil at 14°. Plants of the commercial strain grown under similar conditions were transferred at the same time for controls. The air temperature was the same as that recorded for other experiments conducted at the same time, 14° to 18°. In Table VIII the results obtained at the end of 10 days are compared with the results obtained with seedlings which had been growing at 26° continuously for 20 days.

Because the number of plants used in these tests was small, the infection of one or two more plants in one test than in another made a considerable difference in the percentage of yellows. Even so, the results are consistent enough to indicate that the Wisconsin Hollander plants are more resistant after 20 days than when younger. In Table VIII it may be seen that there is an average difference of 54 per cent of yellows between the older plants of the Wisconsin Hollander and the Commercial Hollander strains at 26°C., while with the younger plants there is a difference of only about 14 per cent. At the same temperature the difference in the percentage of plants killed is equally striking. With the younger plants there is a difference of only 18 per cent, while with the older ones it amounts to 67 per cent. The disease developed very slowly in the Wisconsin Hollander seedlings, and at the conclusion of the experiment most of the affected plants showed only slight symptoms of yellows, while the reverse condition was manifest in plants of the commercial strain.

These investigations were carried further by planting both kinds of seed in sterilized soil and later transplanting the seedlings to "sick" soil. The plants for the first experiment were grown for 30 days in sterlized soil in flats. The temperature of both air and soil ranged from 18° to 22°C. during this period. In the first experiment, which was conducted during May and June, 1918, only three different soil temperatures were used in the series, but the complete series of temperatures from 14° to 35° was employed for the second. Three plants were set in each receptacle, and after transplantation on May 10 the receptacles were exposed to room temperature (18° to 22°) for three days in order to give the plants an equal chance to recover from the shock of being transplanted. The surface of the soil was covered with mineral wool and the receptacles were placed in the tanks at 15°, 17°, and 26°. The final results, recorded 30 days later, are given in Table IX. The air temperature during this experiment ranged from 18° to 22°, with a sudden rise of 10° to 15° for a few hours during the middle of the day. A contrast of the susceptible plants grown in "sick" soil at 15° and at 17° is shown in Plate 2, D, E.

TABLE VIII.—*Influence of age of cabbage seedlings upon the occurrence of yellows*

Date planted.	Condition of soil.	Wisconsin Hollander.						Commercial Hollander.					
		Grown in "sick" soil 20 days from seed, at 26° C.			Grown in "sick" soil 20 days at 14° C., then transferred to 26° for 10 days.			Grown in "sick" soil 20 days from seed, at 26° C.			Grown in "sick" soil 20 days at 14° C., then transferred to 26° for 10 days.		
		Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.
Feb. 1, 1918	Naturally infested	20	80	50	10	50	0	19	78	56	9	89	44
Mar. 29, 1918	do	10	70	20	10	30	10	10	100	50	10	100	100
Average results of two experiments.	Naturally infested soil	30	75	35	20	40	5	29	89	53	19	94	72
Mar. 29, 1918	Artificially inoculated	10	100	100	10	50	10	.....	.....	.....	.....	.....	.....
May 5, 1919	do	.....	.....	.....	.....	.....	.....	18	100	100	18	100	100

TABLE IX.—*Number of cabbage plants which showed yellows at the end of 30 days after transplantation to "sick soil"*

Temperature.	Number of plants.	Wisconsin Hollander.			Commercial Hollander.		
		Number healthy.	Number yellow.	Number dead.	Number healthy.	Number yellow.	Number dead.
°C.							
14	3	3	0	0	3	0	0
17	3	3	0	0	0	3	2
26	3	3	0	0	0	3	3

All of the Wisconsin Hollander plants remained healthy throughout this experiment, whereas all of the susceptible commercial plants at 17° and 26° C. developed typical yellows symptoms. At 26° yellows began to appear after 11 days and at 17° after 17 days. All three plants at 26° were dead after 17 days.

In the second experiment, plants were grown in sterilized soil 36 days from seed before they were transplanted into "sick" soil on May 24, 1919. The air temperature ranged from 22° to 25° C. during this period, but the subsequent treatment was similar to that in the preliminary experiment. The final results, recorded on June 13, are shown in Table X.

TABLE X.—*Number of cabbage plants which showed yellows at the end of 20 days after transplantation to "sick" soil*

Temperature.	Wisconsin Hollander.			Commercial Hollander.		
	Number healthy.	Number yellow.	Number dead.	Number healthy.	Number yellow.	Number dead.
°C.						
14	3	0	0	3	0	0
17	3	0	0	2	1	0
20	3	0	0	0	3	0
23	2	[a] 1	0	0	3	2
26	1	[a] 2	0	0	3	3
29	1	[a] 2	0	0	3	3
32	2	1	0	1	2	0
35	3	0	0	0	3	0

[a] The Wisconsin Hollander plants showed yellows in none but the lower leaves, and there only in incipient form.

Here again, all three of the Commercial Hollander plants developed typical yellows at 23° to 29°, inclusive, whereas only one or two of the Wisconsin Hollander plants showed any symptoms of the disease at all and then only in incipient form in the lower leaves. The number of plants is too small to warrant definite conclusions, but the evidence from both field and greenhouse experiments seems sufficient to indicate that older plants are more resistant than younger ones, and also that resistance becomes more stable as the plants grow older. There is also some indication here that resistance was weakened by starting the plants at a higher temperature, but the experiment needs to be repeated with larger numbers of plants before final conclusions on this point are justified.

The fact that a higher percentage of yellows develops at temperatures unfavorable for the growth of the seedlings is further evidence that the resistance is "broken down" at these temperatures. It has been reported that hereditary characters in several different organisms have been modified by changing the environment (including temperature) of the individuals. Baur (*3, p. 8–9*) found that flower color, an inherited character of the Chinese primrose (*Primula sinensis rubra*), was conditioned upon temperature. The plants were grown by the usual method until about one week before the blossoming stage; then some of them were put into a warm room (30° to 35° C.) and the others into a cool room (16° to 20°.) The plants at the higher temperature produced pure white flowers, while those at the lower temperature produced flowers of the normal red color of the variety. Biffen (*4*), in his work on the inheritance of resistance to yellow rust in wheat, concluded that any factor altering in any way the metabolic processes of the plant in turn alters the degree to which it is attacked by yellow rust and probably other fungi as well. He also found that a variety of wheat which under ordinary conditions of cultivation would be classed as moderately susceptible may be severely attacked when large amounts of nitrates are added to the soil.

FIELD EXPERIMENTS

FIELD OBSERVATIONS

Jones and Gilman (*11*) first recorded the fact that severe attacks of cabbage yellows are associated with hot, dry weather. Gilman (*8*) analyzed this evidence in detail and showed that the incubation period of the disease under field conditions is also materially influenced by soil temperature. The incubation period was about 14 days in 1912 when the mean daily temperature 6 inches below the surface was about 23° C. and 20 days in 1914 when the mean daily temperature at the same depth was about 20°.

Similar observations were made during the summers of 1917 and 1919 in the same field by the writer. In 1919 the mean daily soil temperature 4 inches below the surface was 25° C. at the time of transplantation. Thirteen days later 30 per cent of the Commercial Hollander plants showed typical yellows. This high percentage of diseased plants, together with the different stages of symptoms at that time, indicated that the disease had been present in some of the plants at an earlier date. According to these observations, the incubation period of the disease may vary under field conditions from about 12 days with a

mean soil temperature of about 25° to 20 days with a mean soil temperature of about 20°. It has also been repeatedly observed (*11*, *8*) that the plants which escape or survive the disease for the first month may remain healthy during the remainder of the season.

These observations gave sufficient evidence to prove that soil temperature is an important factor in the development of yellows in cabbage plants transplanted into "sick" fields. However, they do not satisfactorily show what results might be obtained with younger seedlings planted at intervals throughout the season. Data upon experiments of this nature were considered of distinct value for comparison with results obtained in the greenhouse at different temperatures. Therefore, experiments were begun in 1917 for obtaining such data. Both the resistant Wisconsin Hollander and the susceptible Commercial Hollander were used. By beginning to plant early in the season and continuing until late in the summer, it was possible to have each successive crop of seedlings exposed to a different range of soil temperature. It was the original plan to plant the seed at intervals of 7 to 14 days and record the final data 20 days later, as was done in the greenhouse, but for various reasons this schedule could not at all times be very closely adhered to. Consequently, the data obtained in these experiments are not altogether comparable with those obtained in the greenhouse. Even so, as will be shown later, the two sets of data coincide closely enough to justify the conclusion that under field conditions soil temperature is one of the chief limiting factors in the development of yellows in young seedlings as well as in the older plants after transplantation.

EXPERIMENTAL METHODS

The soil temperature was recorded in 1917 in the experimental plot at 1 inch below the surface throughout the growing season and at 4 inches for a part of the season. In 1919 the temperature was recorded only at 4 inches below the surface. Fries kerosene bulb thermographs were used for this purpose. The instruments were checked once a week against a standardized thermometer to insure accuracy.

The seed was sown in short rows and observations were made every few days for a period of 20 to 30 days. In a few cases more than 30 days intervened between observations. During the driest part of the season it was necessary to water the soil at the time the seed was planted in order to insure germination. The seedlings were counted soon after they emerged from the soil, and this number was used as a basis for calculating the percentage of disease. The results of the experiments of 1917 and 1919 are given in summarized form in Table XI; the percentage of yellows and the range of the soil temperature are shown graphically in figures 7 and 8. In these figures is shown the correlation between the percentage of yellows and the curves which represent the range of mean daily soil temperatures. The mean is an average of the temperature readings taken at 2-hour intervals during the day. This is only a relative temperature, of course, because it gives no notion of the duration of the extremes, which is undoubtedly an important factor with diseases of this type.

EXPERIMENTAL DATA.—An inspection of Table XI and figures 7 and 8 shows that seedlings started early in the season may remain absolutely free of the yellows disease for the first 30 days, while the successive later plantings, up to about August 15, show a progressive increase in

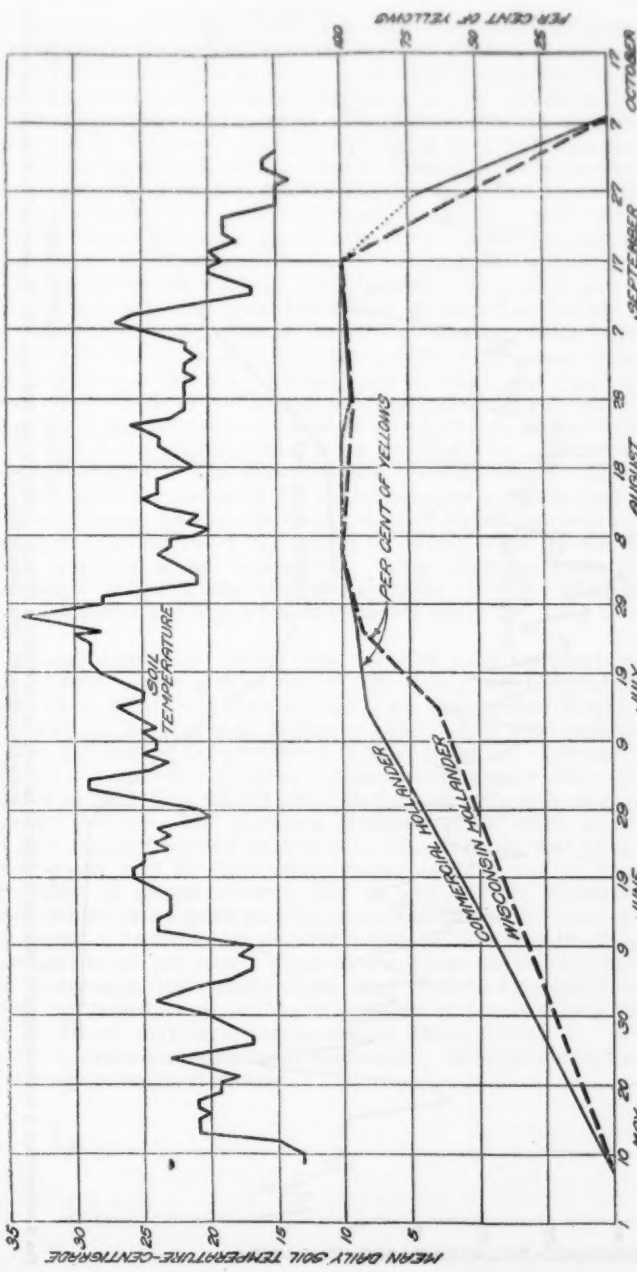

FIG. 7.—Correlation of the development of yellows in Commercial Hollander seedlings in the field, 1912, with the mean daily soil temperature 1 inch below the soil surface. Data given in Table XI.

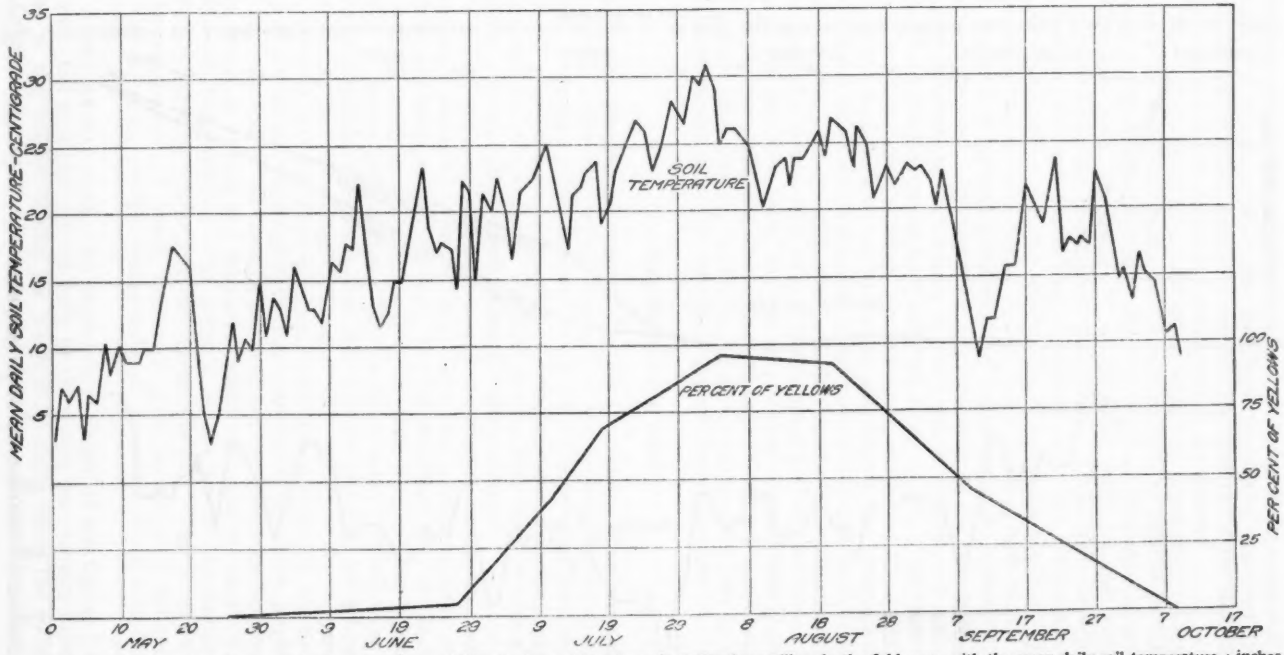

FIG. 8.—Correlation of the development of yellows in Commercial Hollander and in Wisconsin Hollander seedlings in the field, 1919, with the mean daily soil temperature 4 inches below the soil surface. Data given in Table XI.

the percentage of disease. Plantings made after the latter date showed a rapid decrease in percentage of disease until about September 15, after which the disease did not develop. It may also be observed (fig. 7 and 8) that this rise and fall in the percentage of disease correlates in general with the soil-temperature curve. The disease did not appear in the early part of the season until the soil temperature had remained above 17° C. for several days, and it ceased to develop in the latter part of the summer as soon as the temperature dropped below this point. By comparing the temperature curves in figures 7 and 8, it may be observed that the soil did not warm up as early in 1917 as in 1919 and that the development of yellows was deferred in a corresponding manner.

During the hot, dry period, which included the greater part of July and the first half of August, the disease developed as quickly as, and even more destructively than, at the high temperatures in the greenhouse. This result was probably due to the combined influences of high soil temperature and low soil moisture, each of which was found to favor rapid development of the disease under greenhouse conditions. On the other hand, the disease was found to develop more slowly in the field at the lower temperatures than in the greenhouse at constant similarly low temperatures. This difference was probably due to the effect of the wide daily fluctuation of the soil temperature upon the fungus under field conditions—that is, the short period of higher temperature might have been insufficient for the fungus to recover from the effect of the longer duration of low temperature. Often when the mean temperature was about 20° C. the range was probably below 20° all night and a large portion of the day, while it remained above the mean only a few hours.

In several instances, during both 1917 and 1919, the plants were left in the beds for the greater part of the season and observations were made upon them from time to time. By this method it was learned that most of the Wisconsin Hollander plants which were healthy at the end of the first 30 days remained so throughout the experiment. The number of plants thus escaping the disease was greater when they were started in May than when started in July or in August when the soil was hot and dry. The Wisconsin Hollander plants which were started on May 15, 1917, showed no yellows at the end of the first 33 days, and only 15 per cent 62 days after planting. There was no increase in percentage of disease between July 18 and July 26. Conversely, the plants which were started on July 14, 1917, showed 96 per cent of yellows on August 4, 21 days after planting. Sixteen days later the disease had increased to 98 per cent. These data confirm the results obtained in the greenhouse, that plants which were permitted to establish themselves before being exposed to the Fusarium at a temperature favorable for its attack were more resistant than plants started in "sick" soil at the optimum temperature for the fungus. In other words, the plants became more resistant with age.

TABLE XI.—*Percentage of plants developing yellows when planted in the field on different dates during the season*

Date of planting.	Date of observation.	Number of days between time of planting and final observation.	Total number of plants.		Percentage of yellows.	
			Wisconsin Hollander.	Commercial Hollander.	Wisconsin. Hollander.	Commercial Hollander.
1917.						
May 25	June 27	33	100	100	0	5
June 21	July 11	20	100	100	15	57
June 27	July 18	21	100	100	22	70
July 14	Aug. 4	21	140	104	96	97
July 26	Aug. 20	25	89	205	94	94
Aug. 18	Sept. 8	21	400	120	48	48
Sept. 22	Oct. 8	16	100	100	0	0
1919.						
Apr. 6	May 8	32	200	200	0	0
May 8	June 12	35	200	200	35	45
June 18	July 14	26	256	173	64	92
June 30	July 25	25	191	96	94	96
July 14	Aug. 6	23	111	93	100	100
July 30	Aug. 22	23	269	140	97	100
Aug. 6	Aug. 28	22	210	266	96	97
Aug. 12	Sept. 16	35	390	360	100	100
Aug. 20	Sept. 16	26	28	131	100	100
Sept. 7	Sept. 27	20	152	134	53	70
Sept. 16	Oct. 8	22	213	280	0	0

INFLUENCE OF SOIL MOISTURE UPON THE OCCURRENCE OF YELLOWS IN CABBAGE SEEDLINGS

EXPERIMENTAL METHODS

The experiments for measuring the influence of soil moisture upon the occurrence of yellows were conducted during the winter of 1918 and the spring of 1919 simultaneously with, and under the same atmospheric conditions as, the soil-temperature experiments. The same kind of soil and receptacles were used, but no cinders were placed on the bottom of the receptacles. The moisture-holding capacity of the soil was found by means of 5 by 20 cm. tubes to be 46 per cent. When based upon wet weight, this gave approximately 31 per cent moisture.

The moisture content of the soil was kept constant during the experiments through the use of one Livingston cylindrical auto-irrigator, 5 by 15 cm., in each receptacle. The cup was imbedded vertically in the soil, and the water reservoirs were so placed that the moisture content became adjusted at 14.5, 19, 23, and 26 per cent, respectively, in the four sets of duplicate receptacles. The receptacles with 14.5 per cent moisture were kept in the series for only about two weeks when the water columns broke and they were not restored in time to keep them in the series. Moisture determinations were made just before the seed was planted and at the conclusion of the experiment. The percentage of moisture at the conclusion was only a few tenths of 1 per cent lower than at the beginning. The soil temperature was kept constant at 22° to 23° C. throughout the experiment.

After the soil moisture had become constant, seed of the Commercial Hollander strain was planted in the usual manner, and the final data were recorded 28 days later. A few days after the plants emerged from the soil, they were thinned to the desired stand and a half-inch layer of mineral wool was placed over the surface of the soil to equalize surface temperatures. In Table XII the average results from the duplicate receptacles are shown separately for each experiment at the end of different periods of time. The average results of the two experiments are given in Table XIII and shown graphically in figures 9 and 10.

TABLE XII.—*Influence of soil moisture upon the development of yellows in cabbage seedlings, shown at end of different periods after seeding*

FIRST EXPERIMENT, BEGUN FEBRUARY 28 AND CONCLUDED MARCH 27

Number of days after planting.	19 per cent moisture.			23 per cent moisture.			26 per cent moisture.		
	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.
10	20	0	0	20	0	0	20	0	0
12	.....	20	0	.....	0	0	.....	3	0
15	.....	50	0	.....	5	0	.....	20	0
17	.....	85	30	.....	45	0	.....	35	5
20	.....	95	50	.....	80	15	.....	70	25
24	.....	95	95	.....	95	55	.....	95	60
28	.....	100	95	.....	100	80	.....	100	75

SECOND EXPERIMENT, BEGUN APRIL 23 AND CONCLUDED MAY 22

10	17	0	0	27	0	0	31	0	0
12	.....	40	20	.....	0	0	.....	3	0
15	.....	70	30	.....	4	0	.....	52	0
17	.....	70	40	.....	15	0	.....	65	0
20	.....	80	60	.....	63	7	.....	81	29
24	.....	100	60	.....	63	15	.....	87	42
28	.....	100	90	.....	78	48	.....	94	61

TABLE XIII.—*Percentage of cabbage plants developing yellows in soil with different moisture contents—average results of the two experiments shown in Table XII*

Number of days after planting.	Percentage of plants yellow.			Percentage of plants dead.		
	19 per cent moisture.	23 per cent moisture.	26 per cent moisture.	19 per cent moisture.	23 per cent moisture.	26 per cent moisture.
10	0	0	0	0	0	0
12	30	0	3	10	0	0
15	60	5	36	15	0	0
17	78	30	50	35	0	3
20	88	72	75	55	11	27
24	98	79	91	78	35	51
28	100	89	97	93	64	68

EXPERIMENTAL DATA.—From Table XIII it may readily be seen that with favorable temperature yellows develops at any percentage of soil moisture permitting growth of the cabbage seedlings. However, it

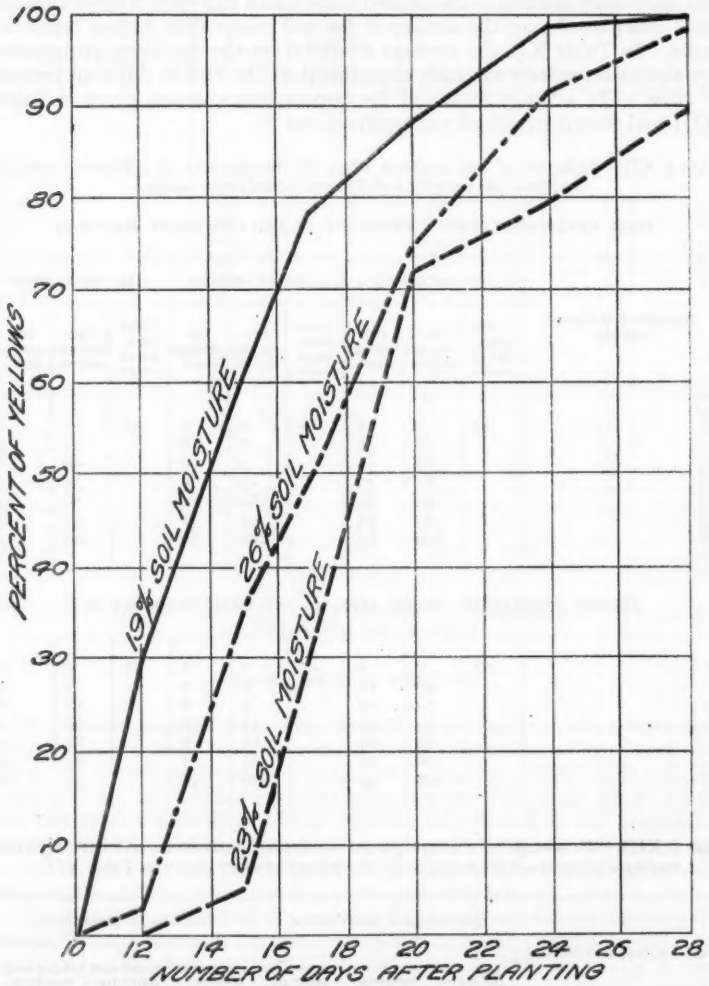

FIG. 9.—Comparison of the rate of development of yellows in Commercial Hollander seedlings given in Table XIII, grown 28 days at 23° C.

developed most rapidly and destructively in soil with 19 per cent moisture, even though, as will be shown later (Table XIV), this percentage of moisture was most favorable for the growth of the seedlings. This was a case, then, where the most vigorous plants were the least resistant to the disease.[5] The development of the disease with 19 per cent moisture

[5] Plants were grown long enough with about 15 per cent soil moisture to show that the yellows developed even more rapidly with this amount than with 19 per cent moisture. This percentage of moisture was about as low as would permit growth of the seedlings.

was very rapid until most of the plants were affected, after which the remaining plants showed infection more slowly. Conversely, the disease developed slowly but at a fairly uniform rate with 23 and 26 per cent moisture, and at the conclusion of the experiment the percentage of

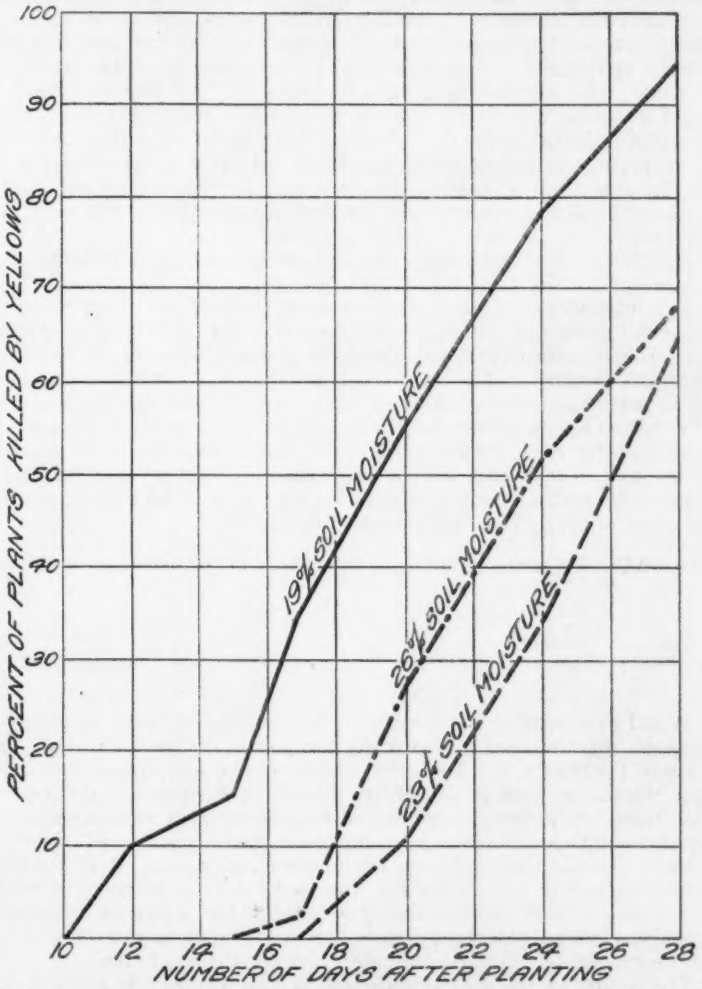

FIG. 10.—Comparison of death rate from yellows in Commercial Hollander seedlings given in Table XIII, grown 28 days at 23° C.

disease was noticeably lower, especially with 23 per cent moisture. It was rather surprising to find that this comparatively slight difference in percentage of moisture made such a significant difference in the rate of development of yellows.

The difference in death rate of the affected plants is even more striking. At the conclusion of the experiment the number of plants killed by yel-

lows at 19 per cent moisture was 29 per cent greater than at 23 per cent moisture and 25 per cent greater than with 26 per cent moisture. At first sight, this condition suggests that because of the reduced functional root system the affected plants were unable to take up the necessary amount of water from the soil with 19 per cent moisture. These results are probably partially explainable on this basis, but the more rapid death rate with 26 per cent moisture than with 23 per cent and the general appearance of the plants in the former case suggest the operation of some factor in addition to deficient water supply. The fact that beyond a certain point both the general vigor of the highly susceptible plants and the percentage of yellows in them decreased with an increase in percentage of soil moisture suggests a reduction in the virulence of the Fusarium. It is possible, therefore, that the reduced oxygen supply in the soil due to increased water content affects both host and parasite.

INFLUENCE OF SOIL MOISTURE UPON THE GROWTH OF CABBAGE SEEDLINGS

It is interesting to note in this connection the influence of soil moisture upon the growth of cabbage seedlings themselves. This investigation was made simultaneously with the study of the influence of soil moisture upon the occurrence of yellows. The dry weight of the plants was used as an index of growth in this case, as previously, and the plants grown in sterilized soil as controls for the second test in the study of the disease were used for the determinations. The plants were cut at the surface of the soil 35 days after seeding and dried for 16 hours at 95° C. Six plants from each of the two receptacles were used. The average weight per plant of the 12 plants is given in Table XIV.

TABLE XIV.—*Dry weight of cabbage seedlings grown in soil with different moisture contents*

Soil moisture (per cent)	19	23	26
Dry weight per plant (gm.)	0.478	0.343	0.251

It will be seen from the foregoing table that the soil with 19 per cent moisture was the most favorable for increase in dry weight. Also, the vigor of the plants, rich leaf color, and extensive root system indicated that the plants were in a healthy, flourishing condition. Contrasted with these, the plants growing in soil with 26 per cent moisture showed decided stunting, yellowish-green leaf color, and a greatly reduced root system. In addition, the roots were brown and small. Both the discoloration and the disintegration were even more pronounced in roots grown in the "sick" soil, probably because of the action of secondary organisms following the Fusarium. The plants grown in the soil with 23 per cent moisture exhibited characters between these extremes.

The results of these experiments show that cabbage is not a high-moisture-loving plant and that the Fusarium is more pathogenic in soil with low moisture content.

SUMMARY

(1) Cabbage yellows, a disease caused by the vascular parasite *Fusarium conglutinans*, has been observed to develop in its most destructive form in southeastern Wisconsin only under conditions of hot, dry weather,

whereas there is little or no development even in the "sickest" soils during moist, cool weather.

(2) Hot, dry weather also retards the growth of cabbage plants.

(3) During such critical periods they become pale green in color and show a general lack of vigor; even the resistant Wisconsin Hollander shows a considerable percentage of incipient yellows. With the return of favorable rainfall and lower temperature the resistant plants overcome the attack of yellows and produce marketable heads.

(4) Such field observations soon convince one that the occurrence and severity of yellows are closely correlated with the influence of soil temperature and soil moisture, and the question arises as to how far and in what way these influences relate to the host on the one hand and to the parasite on the other.

(5) The commercial varieties have shown considerable differences in degree of resistance to Fusarium. Also, within any one variety there is always a variation in resistance as between individuals. During severe seasons most of the plants of the standard commercial varieties quickly succumb to the disease; others linger along in a dwarfed condition, slowly shedding their lower leaves, whereas a few scattered individual plants in the field usually remain healthy and produce marketable heads. This variation in individual susceptibility or disease resistance has been the basis for developing the resistant Wisconsin strains.

(6) The preceding observations and experiments were made upon plants which had been transplanted into the field.

(7) Gilman (*8*), in his greenhouse experiments, showed that yellows did not develop below 17° C. while it developed quite destructively at 23° to 25°. He also studied the effect of certain temperatures upon the growth of the fungus in culture, but he did not define the upper temperature limits either for its growth or for the occurrence of the disease.

(8) The writer undertook to learn more exactly the factors regarding these questions, using seedling cabbage plants. The purposes outlined were: (1) To determine the range of soil temperature for the occurrence of yellows in cabbage seedlings, the air temperature being kept constant; (2) to study the influence of soil temperature upon the growth of cabbage seedlings in noninfested soil; (3) to determine in like manner the influence of soil moisture both upon the growth of cabbage seedlings and upon the occurrence of yellows in them; (4) to study the influence of high soil temperature and soil moisture upon the susceptibility of the resistant strain, that is, upon the "breaking down" of resistance. These experimental investigations have justified several conclusions bearing upon these questions as follows.

(9) Pure cultures of *Fusarium conglutinans* on potato agar plates showed the following relations to temperature: (1) The organism grew at temperatures ranging from 7° to 35° C.; (2) the optimum temperature, using diameter of colony as a criterion, at the end of 7 days was 25° to 27°; (3) although no growth took place in 7 days at 37°, the organism was not killed at this temperature.

(10) Cabbage yellows develops in seedlings growing in "sick" soil at soil temperatures ranging from 17° to 35° C. At 17° it develops very slowly even in the most susceptible strains. In naturally infested soil the disease appears first and develops most rapidly in both resistant and susceptible strains at 26° to 29° and in sterilized artificially inoculated soil at 29° to 32°.

(11) It thus appears that the optimum temperature for the vegetative growth of the fungus in culture practically coincides with the optimum for the development of yellows in seedlings. This is above the optimum for the growth of the host plant.

(12) Cabbage seedlings grew at all temperatures from 14° to 38° C., but only very poorly at the latter. At 38° the seedlings emerged from the soil, but most of them died before developing any true leaves. The optimum soil temperature for seedling growth was found to be about 20° when the Wisconsin tank method was used.

(13) Soil temperature greatly influences the length of the incubation period for the disease. Under controlled conditions the incubation period varied from 18 days at 17° C. to 8 days at 29° to 32°.

(14) With constant favorable temperature yellows developed at any percentage of soil moisture permitting growth of cabbage seedlings. In soil with a moisture-holding capacity of 46 per cent the yellows developed more rapidly and destructively in susceptible plants when the moisture was held at 15 per cent than at 19, 23, or 26 per cent. At 19 per cent moisture the disease appeared two days later than at 15 per cent, but once it had started the subsequent rate of development was about the same in both cases.

(15) Nineteen per cent soil moisture was the most favorable for the growth of cabbage seedlings when the soil temperature was held at 23° C. and the air at 14° to 18°. The growth of plants was materially checked at 26 per cent and also at 15 per cent soil moisture. At these less favorable moistures the color of the foliage was quite abnormal. Plants grown at 19 per cent soil moisture had healthier color and a more extensive root system, and the dry weight was almost twice that at 26 per cent moisture. Thus it is evident that the soil moisture (15 per cent) which was too low for good growth of the host plant was most favorable for the development of yellows, while the soil moisture (19 per cent) which proved almost equally as stimulating to the disease was highly favorable for normal development of the host plant.

(16) The preceding conclusions relative to the relation of soil temperature and soil moisture to the occurrence of yellows in cabbage seedlings were first worked out in the greenhouse under experimentally controlled conditions.

(17) Soil temperature and soil moisture influenced the occurrence of yellows in the field in a manner similar to that in the greenhouse. With seedlings started in May, June, and September when the soil temperature was low, the disease showed a lower percentage and was slower in appearing than with those started in July and August when a high soil temperature prevailed.

(18) These facts, no doubt, are of significance in the geographical distribution of the disease. In the Southern States, where cabbage is generally grown commercially as a winter or early spring crop, the soil temperature is very probably too low for the organism to gain a foothold on such plants. Reports from these sections indicate, however, that where once introduced the Fusarium establishes itself on summer-grown cabbage or other related hosts and may be expected to persist and attack the crops whenever soil temperature is favorable.

(19) The foregoing conclusions are based on the general or average behavior of the cabbage seedlings. It is in this connection that noteworthy variations in the incubation period as between certain individual plants in the same receptacle may be as great as between some plants

grown at 17° and at 29° C. This variation occurs not only with seedlings of the susceptible strain but also with those of the Wisconsin Hollander.

(20) The great difference in the individuality of plants, as shown by the variation in the length of incubation period of the disease and degree of infection of plants, is conclusive evidence of a variation in degree of resistance. This variation in degree of resistance is, no doubt, due to a lack of factors or to a heterozygous condition of some of the plants for the factors for resistance.

(21) The degree of resistance shown by a strain of cabbage depends to a considerable extent upon the environmental conditions under which the plants are grown. In our trials all plants remained healthy in "sick" soil below 17° C.; many were resistant at 17° to 23°, and a small number were resistant at the higher temperatures. At all temperatures within the infection range some plants showed intermediate degrees of resistance, whereas others were entirely susceptible. Somewhat similar results were obtained with different percentages of soil moisture.

(22) Fusarium resistance in cabbage becomes more pronounced with increasing age of the plant. Young seedlings of the resistant strain, Wisconsin Hollander, developed a relatively high percentage of yellows when started in "sick" soil at high temperatures, whereas plants grown for 30 days or more in noninfested soil, or even in "sick" soil below 17° C., when transferred to "sick" soil at high temperatures developed only a low percentage of yellows and then usually only in an incipient form.

(23) This fact has practical significance in predicting, interpreting, or improving the performance of this and the other resistant Wisconsin strains in the following ways:

(*a*) In the first place, it indicates that the strains will give the best results commercially when started in a noninfested seed bed during cool spring weather. This accords with the best practice of commercial cabbage growers in the Northern States.

(*b*) In the second place, it indicates that these resistant strains may safely be recommended for trial in Fusarium "sick" soil in all geographic localities where the prevailing temperatures at the different early stages of development of the cabbage plants are not distinctly higher than those in Wisconsin.

(*c*) Finally, the resistance of these Wisconsin strains may be expected to "break down" in some degree proportional with the elevation of temperature above this point. However, in the Northern States, even in the warmer seasons, this usually stops with the incipient stages of the disease and leaves the crop commercially successful. If more trying conditions are met with elsewhere, it seems probable that through further selection strains showing a correspondingly higher degree of resistance may be secured.

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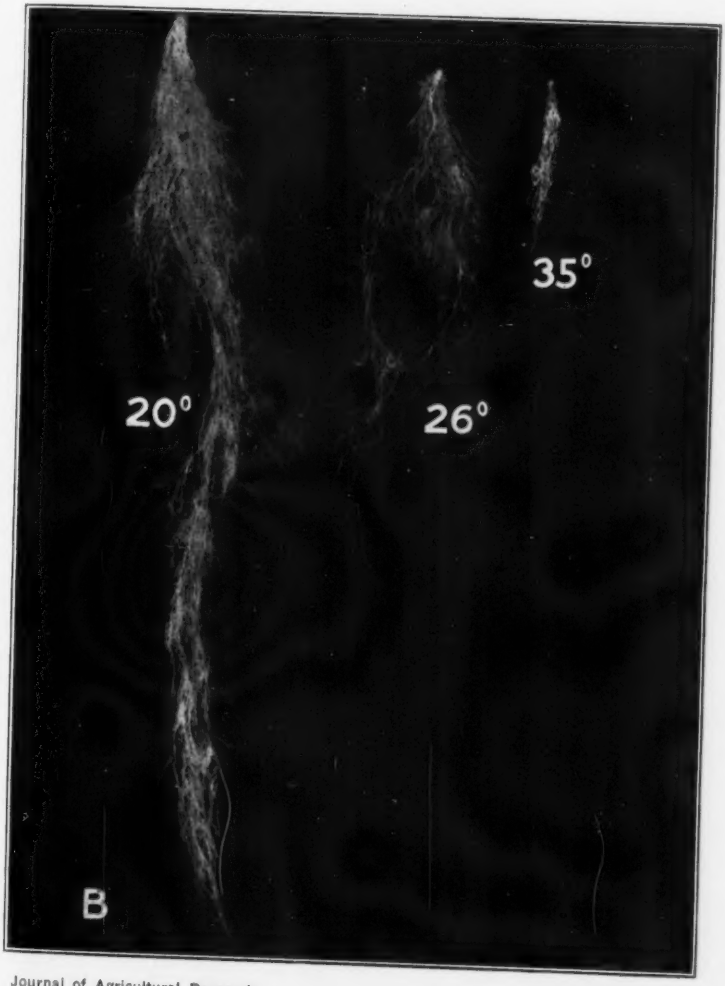
PLATE 1

A.—Comparison of shoots of Wisconsin Hollander cabbage seedlings grown during November and December for 53 days from seed in noninfested soil at the temperatures indicated.

B.—Comparison of root systems of plants shown in A.

A
20°
26°
35°


35°
20°
26°
B

A
SUSCEPTIBLE.
STERILE SOIL.
HEALTHY.
B
SUSCEPTIBLE.
SICK SOIL.
DISEASED.

C
RESISTANT.
SICK SOIL.
HEALTHY.

D
SUSCEPTIBLE.
SICK SOIL.
15° HEALTHY.
E
SUSCEPTIBLE.
SICK SOIL.
17° DISEASED.

PLATE 2

A.—Commercial Hollander plants grown 30 days from seed in sterilized soil at 17° C. All 10 plants healthy.

B.—Commercial Hollander plants grown 30 days from seed in "sick" soil at 17° C. Three of the 10 dead; all others yellowing and stunted.

C.—Wisconsin Hollander plants grown 30 days from seed in "sick" soil at 17° C. All healthy.

D.—Susceptible Commercial Hollander plants grown 36 days from seed in non-infested soil, then 30 days after transplanting to "sick" soil at 15° C. All healthy.

E.—Plants of same variety as those in D and grown under same conditions but transplanted to "sick" soil at 17° C. Two dead; the other yellow and stunted.

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ACTION OF SOAP UPON LEAD ARSENATES[1]

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Soap is sometimes added to the water used for applying lead arsenate as an insecticide. Few authorities advise its use on apple trees or tender vegetation, but it is occasionally advised for use upon hardy and smooth-leaved crops, such as cabbage and sugar beets. It is said that the use of soap with arsenates is increasing.

THE ADVANTAGES

Several advantages are to be gained by the use of soap. The lead arsenate remains in suspension longer in soap solution than in pure water, hence is more easily and evenly applied, especially when hand-operated sprayers are used. The soap also helps to spread the arsenical. J. R. Parker (*3*) [2] found that soap retarded the settling of lead arsenate and stated that it also improved the spreading upon smooth-leaved plants.

THE DISADVANTAGES

The disadvantage to be feared is that of burning the leaves of the crop sprayed by arsenic dissolved by the action of the soap upon the lead arsenate.

Tartar and Bundy (*6*) in 1913 reported that fruit trees were injured by spraying with soap and lead arsenate and showed that the use of the soap increased the quantity of soluble arsenic in the liquid. They also noted that acid arsenate was much more soluble in soap solution than neutral arsenate. Headden (*1*) had already pointed out the danger in using water containing alkali salts for arsenical sprays. It was therefore natural that some presumed that the solubility of lead arsenate in soap solution was due to free sodium carbonate in the soap, and that the damage could be avoided by using only neutral soaps. However, it has been commonly known to chemists for a long time that lead readily forms insoluble soaps. Therefore it is to be expected that neutral soaps might undergo double decomposition with the lead arsenate, forming lead soap and alkali arsenates which would be soluble. This possible reaction may be represented thus:

Sodium soap + lead arsenate = sodium arsenate + lead soap. As the sodium compounds are soluble and the lead compounds are insoluble, it is not to be expected that the reaction will go to completion in either direction. This double decomposition, however, is not the only imaginable reaction which might take place between the soap and lead arsenate; and, in fact, others have been reported by some investigators and will be discussed later. So far as observed by the writer, no one has heretofore reported whether or not soaps of different fatty acids behaved differently toward lead arsenates.

[1] Accepted for publication May 29, 1922.

[2] Reference is made by number (italic) to "Literature cited," p. 95.

FORMULAS OF LEAD ARSENATES

The composition and naming of lead arsenates is a matter that was at one time in great confusion. It has been held by most chemical workers in this field, however, that the lead arsenates on the market fall into three classes:

1. Triplumbic ortho arsenate ($Pb_3(AsO_4)_2$), called by manufacturers "ortho," "triplumbic," "normal,," or "neutral" lead arsenate.

2. Diplumbic ortho arsenate ($PbHAsO_4$), called "monoplumbic," "diplumbic," "acid arsenate," or simply "lead arsenate."

3. Mixtures of these two compounds.

Tartar and Robinson (*4*, 7) believe that the substances here designated as class 1 are really of much more complicated structure and suggest the formula

$$21Pb_3(AsO_4)_2.Pb(OH)_2.10H_2O.$$

They also state that the diplumbic arsenate, $PbHAsO_4$, can be readily transformed into this basic compound by treating with ammonia and that by this treatment, a definite quantity of arsenic is dissolved and may be recovered in the ammonia solution. It thus is an accurate method for the determination of the quantity of diplumbic arsenate in class 3.

Their formula for this basic compound was refuted in two papers appearing at the same time by McDonnell and Smith (*2*) and by G. Ennis Smith (*5*), both suggesting the same formula, $Pb_5OH(AsO_4)_3$, and G. Ennis Smith suggesting the name lead hydroxy arsenate. By this treatment with ammonia, 40 per cent of the arsenic present in the diplumbic goes into solution and the rest remains insoluble as the basic compound.

All investigators agree that the diplumbic arsenate is the more active compound of the two and that where conditions are such as to cause any apprehension of damage from burning the normal compound should be preferred. On the other hand, the acid arsenate contains a greater percentage of arsenic; hence the consumer can secure the required quantity of arsenic with a smaller number of pounds to pay freight and profits upon. The acid arsenate is sold in much greater quantity in many markets.

G. Ennis Smith, in the paper already referred to (*5*), states that soap acts upon diplumbic arsenate in the way that Tartar and Robinson reported for ammonia (*4*, 7)—transforms it into the more basic lead arsenate and dissolves some of its arsenic but sets free an equivalent quantity of the fatty acid, which is insoluble. Thus assuming that the end product is triplumbic arsenate, the reaction is:

$$3PbHAsO_4 + 2NaC_{17}H_{35}CO_2 = \underset{\text{(insoluble)}}{Pb_3(AsO_4)_2} + \underset{\text{(insoluble)}}{2C_{17}H_{35}COOH} + \underset{\text{(soluble)}}{Na_2HAsO_4}.$$

In this case 33⅓ per cent of the arsenic of the diplumbic arsenate is made soluble. In case we consider the end product to be lead hydroxy arsenate, the reaction is:

$$5PbHAsO_4 + 4NaC_{17}H_{35}CO_2 + H_2O = \underset{\text{(insoluble)}}{Pb_5OH(AsO_4)_3} + \underset{\text{(insoluble)}}{4C_{17}H_{35}COOH} + \underset{\text{(soluble)}}{2Na_2HAsO_4}.$$

In this case 40 per cent of the arsenic of the diplumbic arsenate goes into solution. Under this theory there should be no reaction possible

between soap and the lead hydroxy arsenate; hence treating that compound with soap should not bring arsenic into solution.

McDonnell and Smith (*2*) state that triplumbic arsenate is formed under certain conditions, but that it has a very limited range of existence and is readily changed over to the more basic lead arsenate (lead hydroxy arsenate) by treatment with ammonia. By that change 10 per cent of its arsenic should go into solution; hence that should be the maximum possible to obtain by treatment with soap if the end product is in fact lead hydroxy arsenate.

On the other hand, if the reaction between soap and lead arsenate is of the simple double decomposition type, it might be possible for diplumbic arsenate to yield more than the 33⅓ or 40 per cent above quoted, and it might be that the lead hydroxy arsenate would yield soluble arsenic. Furthermore, whichever the type of the reaction, its completeness might be different with soaps of different fatty acids. With these points in view, the writer's materials were selected as follows:

THE SOAPS

The soaps were prepared in the laboratory from c. p. sodium hydroxid and the best grade of the free fatty acids, stearic and oleic. Palmitic was omitted, as no satisfactory palmitic acid was at hand. After the soaps had been prepared they were analyzed for free alkali and for moisture. The stearic soap contained 83.1 per cent moisture and 0.85 per cent free alkali, computed as sodium carbonate. It was weighed in this form for the first series of trials but is recorded on the dry basis. For the second series this soap was dried to 4.3 per cent moisture and weighed in that form.

The oleic soap contained 83.5 per cent moisture and no free alkali. Though it was not found practicable to dry the oleic soap, on the tables it is reported on the dry basis.

THE LEAD ARSENATES

The lead arsenates were selected from a large number of samples that had been furnished by manufacturers for comparison and are designated in this paper as "M" and "T". "M" was labeled by the manufacturer "Monoplumbic Lead Arsenate" and contained at the time of the first series of tests 41.9 per cent water. When dry, it contained arsenic equivalent to 31.53 per cent As_2O_5, and lead to 65.4 per cent PbO. When received, it was analyzed for water soluble arsenic and found to contain, on the dry basis, 0.85 per cent As_2O_5. This was taken to indicate that this arsenical was a practically pure diplumbic hydrogen arsenate. A later test on the dried material with 4 per cent ammonium hydroxid, in the manner described by Tartar and Robinson (*4*, 7), dissolved 8.85 per cent As_2O_5, indicating that only about 67 per cent of the material was really diplumbic arsenate ($PbHAsO_4$) and that 30 per cent of the arsenic present was in some other form.

"M" was used in paste form for the first series and in dry form for the second.

"T" was labeled "Triplumbic Ortho Lead Arsenate" and contained 46 per cent water, and in the dry material arsenic to the amount of 25.75 per cent As_2O_5 and lead to 72.10 per cent PbO. The water-soluble arsenic when received was equivalent to 0.43 per cent As_2O_5, on the dry basis. This was supposed to indicate that the paste was as

labeled, a pure triplumbic arsenate; but since the authorities already noted cast considerable doubt upon the existence of this salt in commercial lead arsenates, it might have been a mixture. The dry matter when treated with 4 per cent ammonium hydroxid, yielded but 0.22 per cent of soluble As_2O_5, indicating but 1.70 per cent $PbHAsO_4$.

BRINGING THE MATERIALS IN CONTACT

Preliminary work indicates that when the soap solution is mixed with the lead arsenate, either as paste or dry, it is difficult to get concordant results for the quantity of arsenic made soluble. It was also noted that when finely divided lead arsenates were shaken with soap solutions the resulting precipitates were bulky and the individual curds frequently large. Besides, as would be expected of either lead soaps or free fatty acids, they were water-repellent. It seemed probable, therefore, that the particles of lead arsenate, when brought into contact with the soap solution, were at once acted upon at the surface and surrounded with an envelope of the water-repellant reaction product, which in turn protected the lead arsenate at the interior of the curd from further action. This reasoning led to the following procedure.

In each case, the treatment was carried out with 2 liters of soap solution in a 2½-liter acid bottle. The soap to be used for each sample was dissolved in 1 liter of distilled water, to be later mixed with a second liter of distilled water, as follows: The water was placed in the acid bottle and the weighed sample of lead arsenate was placed in a porcelain mortar where it was ground with successive portions of the soap solution. The soap and finely divided material was poured into the liter of water, taking care that the coarser particles were left in the mortar to be ground with the next portion of the soap solution. When all the lead arsenate had become so fine as to pass easily into the water with the soap solution, the remainder of the liter of soap solution was poured into the bottle, making 2 liters. The bottle was stoppered and shaken at intervals for five days, then filtered through paper, using suction. One half the samples were treated as stated; the other half were ground in the water and poured into the soap solution, the only difference being the fluid used for the grinding.

The lead arsenates in the first series were weighed in paste form, 2 gm. of "M" amounting to 0.346 gm. of As_2O_5 and to 0.707 gm. PbO, and 2.15 gm. of "T" amounting to 0.289 gm. of As_2O_5 and to 0.805 gm. PbO.

The soaps were used in two concentrations, 0.6 gm. and 1.8 gm. (dry basis) per 2 liters of solution.

The filtrate thus secured was analyzed for arsenic, while the insoluble material was dried, removed from the filter, and preserved for analysis for lead and for total arsenic.

It was found advisable to remove the soap that remained dissolved in the filtrate. This was accomplished as follows: The measured aliquot to be analyzed was placed in a beaker and a few drops of barium chlorid solution were added, just enough being used to clear the solution. The insoluble barium soap separated at once and was removed by filtering, leaving a clear solution that could be reduced and titrated with iodin in the usual Gooch and Browning method (*8, p. 239*).

The quantity of arsenic rendered soluble and found in the filtrate is given in Table I.

TABLE I.—*Percentage of arsenic made soluble by treatment of lead arsenate paste with soap—first series*

No.	Soap.		Lead arsenate.		As_2O_5 in 200 cc.	As_2O_5 rendered soluble.	Solution in which lead arsenate was ground.
	Quantity (dry).	Kind.	Quantity.[1]	Brand.			
	Gm.		*Gm.*		*Gm.*	*Per cent.*	
1	0.6	Sodium stearate	2.0	"M"	0.01760	50.8	Water.
2	1.8	do	2.0	"M"	.01870	54.0	Do.
3	.6	do	2.0	"M"	.01424	41.1	Soap.
4	1.8	do	2.0	"M"	.01704	49.2	Do.
5	.6	do	2.15	"T"	.00884	32.7	Water.
6	1.8	do	2.15	"T"	.00573	19.8	Do.
7	.6	do	2.15	"T"	.00670	23.2	Soap.
8	1.8	do	2.15	"T"	.00752	26.0	Do.
9	.6	Sodium oleate	2.0	"M"	.00335	9.7	Water.
10	1.8	do	2.0	"M"	.00817	23.6	Do.
11	.6	do	2.0	"M"	.00251	7.3	Soap.
12	1.8	do	2.0	"M"	.00726	21.0	Do.
13	.6	do	2.15	"T"	.00111	3.8	Water.
14	1.8	do	2.15	"T"	.00111	3.8	Do.
15	.6	do	2.15	"T"	.00084	2.8	Soap.
16	1.8	do	2.15	"T"	.00084	2.8	Do.

[1] Pastes contained:

	"M"	"T"
As_2O_5	17.30 per cent.	13.40 per cent.
PbO	35.35 per cent.	37.45 per cent.

TABLE II.—*Percentage of arsenic made soluble by treatment of dry lead arsenate with soap—second series*

No.	Soap.		Lead arsenate.[1]		As_2O_5 in 200 cc.	As_2O_5 rendered soluble.	Solution in which lead arsenate was ground.
	Quantity (dry).	Kind.	Quantity.	Brand.			
	Gm.		*Gm.*		*Gm.*	*Per cent.*	
17	0.6	Sodium stearate	1.35	"M"	0.01280	30.1	Water.
18	1.8	do	1.35	"M"	.02814	66.2	Do.
19	.6	do	1.35	"M"	.01325	31.1	Soap.
20	1.8	do	1.35	"M"	.03097	72.9	Do.
21	.6	do	1.20	"T"	.00625	20.2	Water.
22	1.8	do	1.20	"T"	.01084	35.1	Do.
23	.6	do	1.20	"T"	.00596	19.3	Soap.
24	1.8	do	1.20	"T"	.00893	28.9	Do.
25	.6	Sodium oleate	1.35	"M"	.00144	3.4	Water.
26	1.8	do	1.35	"M"	.00345	8.1	Do.
27	.6	do	1.35	"M"	.00144	3.4	Soap.
28	1.8	do	1.35	"M"	.00308	7.2	Do.
29	.6	do	1.20	"T"	.00122	3.9	Water.
30	1.8	do	1.20	"T"	.00066	2.1	Do.
31	.6	do	1.20	"T"	.00122	3.9	Soap.
32	1.8	do	1.20	"T"	.00081	2.6	Do.

[1] Dry arsenate contained:

	"M"	"T"
Moisture	0	0.24 per cent.
As_2O_5	31.50 per cent.	25.75 per cent.
Lead (PbO)	65.40 per cent.	72.10 per cent.
As_2O_5 soluble in 4 per cent ammonia	8.85 per cent.	0.22 per cent.

The second series was in most respects a repetition of the first, except that the lead arsenates were dry and used in slightly larger quantities per sample. The sodium stearate soap was also almost dry in this case, but the quantity of soaps used (dry basis) was the same as before.

The samples, treatment, and soluble arsenic found are given in Table II.

The grinding in the water or in the soap solution was perhaps more thorough than in the first series, but the shaking was continued for only four days.

It will be noted that the pair of tests 5 and 6, in Table I, are not in agreement with other pairs of similar tests. The general tendency is, the greater the concentration of soap, the greater the quantity of soluble arsenic found, but in this one pair this tendency seems to be reversed. It is believed this indicates some mistake, possibly in the numbering of the bottles, or of the record. The corresponding pair in Table II, No. 21 and 22, is in accord with the tendency noted.

EFFECT OF THE LIQUID USED FOR GRINDING

Comparison of the quantity of arsenic made soluble in pairs of tests otherwise identical, but in one of which the grinding was in the presence of pure water and the other in the presence of soap solution, shows that the quantity of arsenic made soluble is seldom the same. It frequently varies by several per cent of the arsenic present, but does not always vary in the same direction. The cause of this lack of uniformity was not determined, but conceivably might be as follows: The shells of lead soap formed about the small solid particles of lead arsenate are somewhat plastic and the real effect of the grinding might be a "churning" one that would serve to stick some of the particles together and increase the protective action of the shells of lead soap. In other cases the grinding might remove the coating of lead soap, as was the intent when the work was done.

SOLUBLE ARSENIC DUE TO ACTION OF FREE ALKALI DEDUCTED

Since the free alkali in the sodium stearate soap would probably exert some solvent action, it is worth while to compute how much of the soluble arsenic found might be due to that cause. Starting with the reaction

$$Na_2CO_3 + PbHAsO_4 = PbCO_3 + Na_2HAsO_4,$$

the highest ratio possible between sodium carbonate and soluble arsenic pentoxid is 1 to 1.085. On this basis Table III is computed from the data already given, showing the quantity of sodium carbonate present, the equivalent quantity of As_2O_5 that might be made soluble, the quantity of soluble arsenic as actually found, and, by difference, the quantity that must be made soluble by the neutral soap, the quantity of As_2O_5 present in the lead arsenate used, and the percentage made soluble by the action of the soap aside from the free alkali.

Since the oleic soap contained no free alkali, no similar table is presented for correction of the results on oleic soap in Tables I and II.

TABLE III.—*Quantity of As_2O_5 that might be made soluble by the free (excess) sodium carbonate with stearate soap (4.72 per cent)*

No.	Brand of lead arsenate.	Weight of free Na_2CO_3	Equivalent weight of As_2O_5	As_2O_5 found in 2 liters.	Quantity due to soap (by difference).	Total As_2O_5 in system.	Arsenic made soluble by neutral soap.
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per cent.*
1	"M"	0.0095	0.0103	0.1760	0.1657	0.3460	47.89
2	"M"	.0285	.0309	.1870	.1561	.3460	45.12
3	"M"	.0095	.0103	.1424	.1321	.3460	38.18
4	"M"	.0285	.0309	.1704	.1397	.3460	40.37
5	"T"	.0095	.0103	.0884	.0781	.2890	27.02
6	"T"	.0285	.0309	.0573	.0264	.2890	9.13
7	"T"	.0095	.0103	.0670	.0567	.2890	19.62
8	"T"	.0285	.0309	.0572	.0261	.2890	9.02
17	"M"	.0095	.9103	.1280	.1177	.4050	29.06
18	"M"	.0285	.0309	.2814	.2505	.4050	61.85
19	"M"	.0095	.0103	.1325	.1222	.4050	30.17
20	"M"	.0285	.0309	.3097	.2788	.4050	68.84
21	"T"	.0095	.0103	.0625	.0522	.3090	16.89
22	"T"	.0285	.0309	.1084	.0775	.3090	25.08
23	"T"	.0095	.0103	.0596	.0493	.3090	15.95
24	"T"	.0285	.0309	.0895	.0586	.3090	18 96

It is plain from Tables I, II, and III that "M" is very much more acted upon by both soaps than is "T" and that sodium stearate is very much more (two to seven times) effective in dissolving arsenic from lead arsenates than is sodium oleate. It follows that if it is desired to use soap with lead arsenate in spraying, the danger of injury from arsenic in solution can be diminished in some degree by securing triplumbic arsenate but could be almost entirely overcome by using only sodium oleate as the soap.

It is also clear that sodium stearate at least does not act as stated by G. Ennis Smith (5), but dissolves arsenic from the basic arsenate and dissolves much more than the 40 per cent that should be made soluble in transforming diplumbic into lead hydroxy arsenate. This behavior is exactly in accord, however, with the supposition that the reaction is of the double decomposition sort, giving insoluble lead soaps as one of the end products, instead of free fatty acids as Smith states. In order to confirm this supposition, the insoluble residues from the first series were analyzed for arsenic and for lead.

In case the reaction stops as soon as the lead hydroxy arsenate is reached, the ratio of lead oxid to arsenic pentoxid could never be greater than that in the lead hydroxy arsenate, 1 to 3.23, and would usually be lower than that, as there would be some material unacted upon. On the other hand, if the reaction is of the double decomposition sort, it might be possible to carry the reaction far beyond that ratio, as in fact occurs in six of the eight samples that were acted upon by sodium stearate. When oleate soap was used, the reaction was so incomplete that the ratio never approaches even 1 to 3. It may be that soap first transforms diplumbic arsenate into lead hydroxy arsenate and then undergoes a double decomposition with that salt, but it is hard to explain the results on the supposition that the action stops at the lead hydroxy arsenate. The results of the analysis of the dry insoluble residue and the ratio of As_2O_5 to PbO are given in Table IV.

TABLE IV.—*Analysis of 0.1 gm. dry insoluble residue from treatment of lead arsenate with soap solution*

No.	Soap.		Lead arsenate.		Weight of As_2O_5.	Weight of PbO.	Ratio of As_2O_5 to PbO.
	Quantity.	Kind.	Quantity.	Brand.			
	Gm.		*Gm.*		*Gm.*	*Gm.*	
1	0.6	Sodium stearate....	2.00	"M"	0.0101	0.0437	1 : 4.3
2	1.8	.....do.....	2.00	"M"	.0065	.0274	1 : 4.2
3	.6	.....do.....	2.00	"M"	.0122	.0460	1 : 3.8
4	1.8	.....do.....	2.00	"M"	.0065	.0283	1 : 4.3
5	.6	.....do.....	2.15	"T"	.0132	.0475	1 : 3.6
6	1.8	.....do.....	2.15	"T"	.0092	.0310	1 : 3.4
7	.6	.....do.....	2.15	"T"	.0140	.0468	1 : 3.3
8	1.8	.....do.....	2.15	"T"	.0086	.0257	1 : 3.0
9	.6	Sodium oleate.....	2.00	"M"	.0256	.0538	1 : 2.1
10	1.8	.....do.....	2.00	"M"	.0211	.0482	1 : 2.3
11	.6	.....do.....	2.00	"M"	.0322	.0552	1 : 1.7
12	1.8	.....do.....	2.00	"M"	.0204	.0470	1 : 2.3
13	.6	.....do.....	2.15	"T"	.0244	.0635	1 : 2.6
14	1.8	.....do.....	2.15	"T"	.0242	.0610	1 : 2.5
15	.6	.....do.....	2.15	"T"	.0221	.0564	1 : 2.5
16	1.8	.....do.....	2.15	"T"	.0185	.0482	1 : 2.6

Theoretical for diplumbic ortho arsenate ($PbHAsO_4$) 1 : 1.94.
Theoretical for triplumbic ortho arsenate ($Pb_3(AsO_4)_2$) 1 : 2.51.
Theoretical for lead hydroxy arsenate ($Pb_3OH.(AsO_4)_3$) 1 : 3.23.

SUMMARY

The data exhibited make the following conclusions plain:

(1) That pure soaps dissolve arsenic from both samples of lead arsenate and therefore might cause injury to foliage.

(2) That sodium stearate is much more effective in dissolving arsenic from both "M" and "T" than is sodium oleate, from two to six or even seven times as much soluble arsenic being found in the solution of the former as in the latter.

(3) That, as Tartar and Robinson point out (*4*, *7*), the arsenic of diplumbic arsenate is much more acted upon than the more basic compound.

(4a) Increasing the concentration of the stearic soap solution increased the amount of arsenic made soluble.

(4b) Increasing the concentration of the oleic soap made more arsenic soluble from the acid lead arsenate "M" but did not increase the amount made soluble from the basic lead arsenate "T."

(5) The extent of the action was sometimes greater when the lead arsenate was ground in water than in the soap, sometimes less. No definite statement can be made as to which is most effective.

(6) Sodium stearate dissolves far too much arsenic from diplumbic arsenate to confirm the supposition that its action stops with the conversion of diplumbic into lead hydroxy arsenate.

(7) Both sodium stearate and sodium oleate dissolve arsenic from basic lead arsenate, the stearic soap in large quantities (as much as 25 per cent).

(8) Numbers 6 and 7 together indicate that the action of soaps upon lead arsenates is of the double decomposition sort.

(9) Danger of injury from soluble arsenic in spraying with lead arsenates and soap can be largely eliminated if the soap is entirely made from oleic acid.

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